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A study of trace element alterations in total parenteral nutrition solutions using inductively coupled plasma-mass spectrometry

by

Michelle Marie Pluhator-Murton ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of *Master of Science in Experimental Medicine*

Department of Medicine

Edmonton, Alberta

Spring 1996

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DEDICATION

This thesis is dedicated to my husband, Ken, and to my family.
Their love and support have allowed me to reach for the stars.

ABSTRACT

Home total parenteral nutrition (TPN) has been associated with trace element (TE) deficiencies and excesses despite delivery of what is considered to be appropriate amounts of trace elements (TE). This work determined the degree of TE alterations in home TPN solutions subjected to clinically relevant variables, and proposed mechanisms responsible for the inadvertent deviations. *In vitro* studies directed at the three levels of TPN preparation (components, compounding, delivery) were conducted. TE's were analyzed via inductively coupled plasma-mass spectrometry (ICP-MS). Results confirmed the presence of TE contaminants in the component solutions, revealed additional contamination during compounding and delivery, and verified the delivery of adequate amounts of added essential TE's after exposure to clinically relevant variables. Potential mechanisms of TE gains include contamination and leeching from various surfaces in contact with the TPN solution, while those inducing losses include adsorption to surfaces and precipitation of elements from the final solution.

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"Good company in a journey makes the way seem the shorter."–Izaak Walton.

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TABLE OF CONTENTS

PAGE

INTRODUCTION 1

References 5

CHAPTER 1: A SUMMARY OF ESSENTIAL AND NONESSENTIAL TRACE ELEMENTS 8

References 52

CHAPTER 2: TRACE ELEMENT ANALYSIS 68

References 79

CHAPTER 3: INDIVIDUAL COMPONENTS OF THE TPN SOLUTION ARE CONTAMINATED WITH TRACE ELEMENTS 84

References 102

CHAPTER 4: COMPOUNDING EQUIPMENT CONTRIBUTES TRACE ELEMENT CONTAMINANTS TO SOLUTIONS 124

References 132

CHAPTER 5: THE DELIVERY SYSTEM RESULTS IN ADDITION AND LOSS OF TRACE ELEMENTS FROM A TPN SOLUTION 137

References 163

PAGE

CHAPTER 6: SUMMARY AND CONCLUSIONS 184

LIST OF TABLES

TABLE		PAGE
1	Essential and nonessential trace elements	7
2-1	Selected ions and correction equations employed for quantitative analysis of TPN solutions	82
2-2	Comparison of NIST SRM 1643C water with measured values during the study duration	83
3-1	Lot numbers and manufacturers of components used to make a TPN solution	114
3-2	Volume of components used to compose a one litre standard TPN solution	115
3-3	Trace elements examined with the "Spectrum-directed" ICP-MS screen	116
3-4a	Percent contribution of Lot 1 components to the total trace element concentration in a standard TPN solution	117
3-4b	Percent contribution of Lot 2 components to the total trace element concentration in a standard TPN solution	118
3-4c	Percent contribution of Lot 3 components to the total trace element concentration in a standard TPN solution	119

TABLE		PAGE
3-5	Concentration of trace elements from TPN solutions composed of Lot 1 and 2 components relative to the expected concentration in a standard TPN solution	120
3-6	Summary of previously reported trace element contamination of TPN components	122
4-1	Trace elements examined with the "Spectrum-directed" ICP-MS screen	136
5-1	TPN solution used in the early and late delivery experiment	180
5-2	Trace elements examined with the "Spectrum-directed" ICP-MS screen	181
5-3	Comparison of dietary intakes and intravenously delivered concentrations of trace elements stored at various time/temperature combinations	182
6-1	Summary of trace element alterations during compounding and delivery compared to expected concentrations in Lot 1 components	186

LIST OF FIGURES

FIGURE		PAGE
2-1	Components of the Perkin Elmer Sciex Elan 5000A Inductively Coupled Plasma-Mass Spectrometer	80
2-2	Comparison of detection limits in ICP-MS, GFAAS, FAAS, and ICP AES	81
3-1	Concentration of trace element contaminants from the 4mmol/mL NaCl solution	106
3-2	Concentration of trace element contaminants from the 2mmol KCl solution	107
3-3	Concentration of trace element contaminants from the 10% Travasol solution	108
3-4	Concentration of trace element contaminants from the adult MTE-6 solution	109
3-5	Concentration of trace element contaminants from the 10% calcium gluconate solution	110
3-6	Concentration of trace element contaminants from the adult multivitamin (MVI) solution	111
3-7	Concentration of trace element contaminants from the 50% $MgSO_4$ solution	112
3-8	Concentration of trace element contaminants from the sterile water	113

FIGURE PAGE

4-1 The concentration of Zn present as a contaminant in each of the experimental groups 134

4-2 The concentration of Al and B present as contaminants in each of the experimental groups 135

5-1 Outline of delivery experiment 168

5-2 Concentration of zinc delivered in a TPN solution early (36 hours) and late (30 days) after storage at 4°C or 20°C 169

5-3 Concentration of copper delivered in a TPN solution early (36 hours) and late (30 days) after storage at 4°C or 20°C 170

5-4 Concentration of manganese delivered in a TPN solution early (36 hours) and late (30 days) after storage at 4°C or 20°C 171

5-5 Concentration of selenium delivered in a TPN solution early (36 hours) and late (30 days) after storage at 4°C or 20°C 172

5-6 Concentration of boron delivered in a TPN solution early (36 hours) and late (30 days) after storage at 4°C or 20°C 173

FIGURE PAGE

5-7 Concentration of aluminum delivered in a TPN solution early (36 hours) and late (30 days) after storage at 4°C or 20°C 174

5-8 Concentration of barium delivered in a TPN solution early (36 hours) and late (30 days) after storage at 4°C or 20°C 175

5-9 Concentration of vanadium delivered in a TPN solution early (36 hours) and late (30 days) after storage at 4°C or 20°C 176

5-10 Concentration of titanium delivered in a TPN solution early (36 hours) and late (30 days) after storage at 4°C or 20°C 177

5-11 Concentration of strontium delivered in a TPN solution early (36 hours) and late (30 days) after storage at 4°C or 20°C 178

5-12 Concentration of cobalt delivered in a TPN solution early (36 hours) and late (30 days) after storage at 4°C or 20°C 179

INTRODUCTION

Trace elements

Trace elements can be distinguished from major mineral elements in that they exist and function in living organisms in very small concentrations. They typically constitute less than 0.01% of the total body of a human [1]. The term "trace" should not be equated with "unimportant", since these elements perform vital functions necessary for the maintenance of life, growth, and reproduction.

Trace elements are most conveniently classified into two groups: essential and nonessential. A trace element is considered essential if inadequacy consistently results in deficiency symptoms, and repletion successfully reverses the abnormalities. Symptoms may vary from an impairment of biochemical function to ultimately, death. The group of nonessential trace elements includes all others which are present in biological systems but for which no proof of essentiality has been established. Some trace elements, such as Cd, Hg, and Pb are classically considered to be "toxic" elements since they produce deleterious effects when present in very low levels in the body. However, it should be noted that all trace elements, including those considered to be essential, are potentially toxic when the limits of safe exposure are exceeded. There are some trace elements (eg, V, Sn, Si, Ni, As, B, F) which have been accepted as essential in other mammals, but at present there is insufficient evidence of a nutritional requirement for humans. A list of essential and nonessential trace elements appears in Table 1. Such groupings should not be considered as definitive, since it is likely that some of the elements currently considered nonessential will be found to be vital to certain biochemical processes.

In recent years, health professionals have focused increasing attention on the importance of trace elements in the nutritional management of medical, surgical, and trauma patients. Trace elements are necessary components for use in Total Parenteral Nutrition (TPN) therapy because of their biochemical essentiality.

Total Parenteral Nutrition

Parenteral nutrition is the administration of nutrients via the intravascular route. This type of nourishment may serve as a means of either partial or total nutritional support. When patients are unable to use the gastrointestinal tract for the acquisition of nutrients, total parenteral nutrition, TPN, becomes necessary. As implied by its name, TPN may be used as the sole means of feeding a patient. For long term TPN, a permanent central venous catheter made from silicone rubber is placed into the subclavian vein and threaded into the superior vena cava. This allows for the infusion of hypertonic nutritive solutions.

The emergence of TPN in the late 1960's provided the means to support patients who were unable to meet their needs enterally. Similarly, the transfer of TPN to the home provided a safe and effective way of nutritionally maintaining patients who, beforehand, died from malnutrition and infection or spent the majority of their lives in hospital. The majority of home TPN patients consist of those with Crohn's disease, severe short bowel syndrome, radiation enteritis, chronic adhesive obstruction, or diffuse intestinal motility disorders. Such stressed, poorly nourished patients placed on TPN require special needs for nutrients to replace losses and allow optimal nutritional rehabilitation. Long-term home TPN patients must be assured that all essential nutrients, including trace elements, are provided in adequate amounts over periods which may extend into decades. Assurance is also needed that potentially toxic levels of any nutrient, contaminant, or additive are avoided, since parenteral feeding bypasses the normal barriers of the alimentary tract. Despite the delivery of what is thought to be appropriate amounts of trace elements, home TPN has been associated with both trace element deficiencies and excesses.

Many of the initial cases of trace element deficiency during long-term TPN were attributable to a lack of added trace elements to TPN solutions. However, there have been studies which have suggested that trace element deficiency develops as a result of a loss or inactivation of trace element following its addition to the parenteral solution [2]. Any incompatibilities leading to the decreased availability of essential trace elements delivered, can be detrimental to the patient.

There have been studies which have proposed that trace element excess in TPN solutions infused to patients may occur as a result of contamination from the components used to generate TPN solutions [3,4]. The delivery of excess trace elements via contaminated TPN components poses a threat to the individual in the form of a trace element toxicity. Additionally, determination of accurate daily trace element requirements for TPN regimens becomes difficult if the trace element concentration from contaminants is unknown or inconsistent. There have been a number of studies which have attempted to measure the trace element concentration of various TPN components [3,5,6]. Results, indeed, show a large variation in the trace element concentration of TPN components due to contamination [3,7]. Differences in the amount of trace element contamination between manufacturers and between lot numbers of stock from the same manufacturer are apparent in certain solutions [7]. The amount of trace elements present as contaminants can be significant [4]. For example, Al has been found to be a frequent contaminant of TPN solutions [4,8] and toxicity evidenced by high bone, urine, and plasma Al levels have appeared in patients undergoing long-term TPN [9]. Other frequently reported contaminants appearing at variable levels are Zn, Cu, Cr and Mn, which ironically are elements routinely added to TPN solutions [4,10]. There is little existing information regarding the concentration of nonessential trace elements in TPN solutions. Additionally, the various materials, especially plastics, in contact with TPN solutions during their preparation and delivery are made of complex ingredients in which the extent of trace element contamination remains unknown. No studies have specifically and systematically examined the potential for trace element contamination of TPN solutions by the plastic compounding devices and delivery system components employed in modern TPN.

The reports concerning trace element stability in TPN solutions are few. In those that do exist, there is often only speculation as to what may be happening to reduce or increase the amount of trace element present. The literature has revealed variable results from studies examining trace element interactions with glass and plasticware [11]. Conclusions are difficult to ascertain due to variations in TPN solution components, and time and temperature variables. Studies of the past frequently relied upon visual evidence of incompatibility, such as precipitation or colour changes, rather than actual measurement of trace element concentrations [12]. Often the experimental solutions and conditions used to test trace element interactions in TPN solutions were manipulated in ways unlikely to occur clinically. There are very few compatibility studies which have examined modern multielement trace element additives in standard TPN solutions. Few have run clinically relevant TPN solutions containing multielement trace element additives through actual delivery systems under controlled varying conditions, and measured changes in trace element concentration. Recent literature, thus, remains largely suspect of trace element stability and concentrations in TPN solutions.

Study objectives

The mechanisms underlying the delivery of deficient or excess levels of trace elements have not been clearly defined. There is a call for more controlled, clinical experiments of trace element stability and content.

Our hypothesis is that the amount of trace element initially thought to be present in a TPN solution is different than the amount delivered to a patient at the time of infusion.

The objective of this work was, thus, to determine the degree of alteration of trace element concentrations in home TPN solutions subjected to clinically relevant variables, and to propose mechanisms which may be responsible for the inadvertent deviations from the expected concentrations.

The specific aims were: 1) to determine the baseline level of trace elements in each of the components comprising a typical home TPN solution 2) to determine if additional trace elements were contaminating solutions as a result of exposure to compounding devices and 3) to examine the extent of trace element alterations in TPN solutions delivered via a clinically relevant system subjected to typical time and temperature variables. The advanced technology of inductively coupled plasma-mass spectrometry (ICP-MS) was used to measure trace element concentrations for the study.

This study thus reveals the current status of trace element contamination and alterations occurring from the point of stock acquisition, through preparation and compounding, and finally to the delivery of complete TPN solutions prior to entry into the patient. It is hoped that this work will lead to improvements in trace element delivery in home TPN regimens.

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Table 1. Essential and nonessential trace elements

Essential	Nonessential	
	Essentiality not firmly established	No metabolic role assigned
Iron (Fe)	Vanadium (V)	Titanium (Ti)
Zinc (Zn)	Tin (Sn)	Strontium (Sr)
Copper (Cu)	Silicon (Si)	Aluminum (Al)
Manganese (Mn)	Nickel (Ni)	Barium (Ba)
Selenium (Se)	Arsenic (As)	Bromine (Br)
Chromium (Cr)	Boron (B)	Cadmium (Cd)
Iodine (I)	Fluorine (F)	Gold (Au)
Cobalt (Co)		Silver (Ag)
Molybdenum (Mo)		Mercury (Hg)
		Bismuth (Bi)
		Lead (Pb)
		Antimony (Sb)
		Lithium (Li)
		+ 30 others

CHAPTER 1

A SUMMARY OF ESSENTIAL AND NONESSENTIAL TRACE ELEMENTS

This chapter reviews the biochemistry, physiological roles, metabolism, and states of deficiency and toxicity surrounding thirteen trace elements: Zn, Cu, Mn, Se, Cr, B, Al, Ba, V, Ti, Sr, Co, and As. These were elements which were added as part of the essential trace element component for TPN solutions (Zn, Cu, Mn, Se, Cr), or revealed via experimentation undertaken in subsequent chapters to be contaminants of the components, compounding system, and delivery system. Other essential and nonessential elements not directly related to the thesis will not be discussed.

ZINC

Biochemistry

Zinc has been recognized as an essential nutrient for humans since the early 1960's [1,2]. The human body contains 2 to 3 grams of Zn [3,4], and can be found in all tissues in varying concentrations; the highest concentrations occur in the retina, prostate, prostatic secretions, and spermatozoa. Three quarters of the total bodily amount can be found in the skeleton from which it is slowly removed over time [3]. In the blood, most Zn is contained within erythrocytes. A small amount appears in blood serum which contains a Zn concentration of approximately 120 ug/dL [5]. Intracellular Zn accounts for over 95% of the total body Zn [6].

Physiological roles

Zinc is necessary for virtually all aspects of normal cell metabolism. Zinc is an important constituent of more than 20 human metalloenzymes [7,8,9], which are involved in a variety of metabolic activities including carbohydrate, fat, and protein metabolism [10]. Zinc is essential to both DNA and RNA synthesis [2,8,9] where its primary effect is thought to be on the Zn-dependent enzymes that regulate the biosynthesis and catabolic rates of DNA and RNA [9]. Zinc's involvement in the cell cycle is integral to its role in wound healing. Studies have confirmed that Zn accumulates around the wound (where cell division takes place most vigorously), particularly during the initial week of healing [11]. Zinc is necessary for adequate gonadal development and growth response from infancy to adulthood [3]. Zinc plays an important role in pregnancy outcome [12] due to its involvement in cellular growth and maturation, its action as an antibacterial and antiviral agent in amniotic fluid, and the positive correlations which exist between maternal serum Zn concentration and birthweight [13]. Zinc is involved in the action of insulin, thereby affecting glucose tolerance [3,9]. It is thought that Zn participates in the storage of insulin in the B-cells [9]. Zinc is thought to give structure and metabolic stability, or to regulate the tertiary architecture and thus the biological function, of a wide range of macromolecules [14]. Zinc has been suggested to play a role in immune function, since a deficiency of the element can have striking effects on almost all the components of the immune system [15].

Metabolism

Zinc absorption is affected by the amount of Zn in the diet, age, and the presence of interfering substances such as phytate, phosphate, Fe, and Ca. In the case of the average adult, 30 to 50% of the Zn provided daily in the diet is absorbed [4]. Absorption of Zn is thought to occur throughout the small intestine [10]. In the intestinal lumen, Zn is likely matched to a ligand with an affinity for the element (eg, amino acids, pancreatic secretions, organic acids, other ions). The resultant complexes are then presented to the surface of the mucosal cell [3,16]. Events at the surface of the brush border membrane have not been fully defined; however, Zn absorption is though to be a carrier-mediated transport process [10].

Zinc entering the enterocyte equilibrates with the intracellular Zn pool and can be bound by metallothionein and stored until mature cells are sloughed off, utilized in the metabolism of the absorbing cell, or passed through the serosal border to be picked up by the portal bloodstream [9,17].

Newly absorbed Zn is carried in the circulation bound to albumin [18,19], amino acids such as histidine and cysteine, and to macroglobulins and other serum proteins [3,20]. Plasma is the major route of Zn transport within the body. Approximately 30 to 40% of the Zn which enters the hepatic venous supply is removed by the liver, from which it is subsequently released back into the blood [21]. Circulating Zn is incorporated at differing rates into various extrahepatic tissues, all of which have different rates of Zn turnover.

Given normal dietary circumstances, the feces are the major route of Zn excretion. A healthy human adult whose Zn balance is in equilibrium and who ingests 9 to 15 mg Zn per day, excretes approximately 90% of this amount in the feces, with 2 to 10% being excreted in the urine [22]. Increased fecal Zn loses can occur during chronic diarrhea [23]. Increased urinary Zn losses have occurred in certain disease states (eg, diabetes, sickle cell anemia), in burn victims, in severe trauma patients, and in individuals receiving TPN. Sweat losses in healthy adults living in a temperate North American climate have been reported to be from 0.4 to 2.8 mg Zn daily [21]. Menstrual losses of Zn are negligible with average losses of between 0.1 and 0.5 mg Zn per period [21]. Seminal Zn losses are reported as roughly comparable to the amounts lost daily in sweat and urine [24].

The mixed Canadian diet provides approximately 5 mg Zn/1000 kcal [4]. Major dietary sources of Zn are foods of animal origin such as meat, fish, poultry, eggs, and dairy products. Whole grain cereals are also good sources of Zn; however, absorption of Zn from these sources is reduced due to the presence of interfering substances such as phytates and fibre.

A constant need for dietary Zn is necessary to maintain plasma Zn concentrations [25]. The Recommended Nutrient Intake (RNI) for Zn in Canada for adult males and females over the age of 13 years is 12 mg and 9 mg per day, respectively [4]. The RNI is increased during pregnancy and lactation, as well as during periods of growth (eg, infancy).

The Expert Panel for Nutrition Advisory Group, American Medical Association Department of Foods and Nutrition, published its recommendations for the daily intravenous Zn dosages necessary to prevent nutritional depletion. Recommendations for stable adult patients range from 2.5 to 4.0 mg/day. An additional 2.0 mg Zn should be provided to patients in acute catabolic states. For those patients experiencing significant gastrointestinal losses, an additional 12.2 mg of Zn should be added for each litre of small bowel fluid loss or 17.1 mg/kg of stool or ileostomy output [26]. Recommendations for pediatric patients range from 100 to 300 μg/kg/day [26].

Deficiency

Primary Zn deficiency syndrome (ie, that due to inadequate dietary intake and/or poor availability) is characterized by skin lesions, depressed growth, hypogonadism, anorexia and mental lethargy [11]. This condition was first reported in males from the Middle East [11]. Additionally, patients often develop intractable diarrhea, hypogeusia, immunologic impairment, glucose intolerance, alopecia, and anemia [1,27,28]. Secondary Zn deficiency (ie, due to states of decreased absorption, excessive excretion and/or excessive utilization) which can cause further Zn losses, has been documented in the presence of renal disease [29,30], Crohn's disease [31], and in association with burns [32] and alcoholism [33]. Two genetic disorders, acrodermatitis enteropathica (an inborn error of impaired Zn absorption) and sickle cell disease (which involves increased urinary Zn losses) are also associated with suboptimal Zn status [2]. Possible iatrogenic causes of Zn deficiency include use of chelating agents, antianabolic drugs and diuretics [9]. Anti-convulsant drugs, such as Na-valproate have also been implicated in causing Zn deficiency [34]. Failure to add Zn, or sufficient Zn, to TPN has been another cause of deficiency. If left untreated, Zn deficiency is fatal. Symptoms of Zn deficiency rapidly resolve after Zn therapy is initiated.

A number of indices for measurement of Zn status (eg, plasma, hair, and urinary Zn) have been employed clinically. However, none of the methods currently used can be recommended due to the numerous problems affecting their use and interpretation. Presently, the most reliable method for diagnosing Zn deficiency is a positive response to Zn supplementation [35,36].

Toxicity

In order for overt symptoms of Zn toxicity to develop, a relatively large amount of Zn must be ingested. Most reports of acute toxicity have been related to food poisoning incidents. An emetic dose of approximately 1 to 2 g $ZnSO_4$ (corresponding to 225 to 450 mg Zn), presumed to have leached into a beverage stored in a galvanized container, has been reported to have caused toxic symptoms of nausea, vomiting, epigastric pain, abdominal cramps and bloody diarrhea [3,37,38]. Other reports of Zn toxicity have been associated with inhalation of Zn-chloride from industrial pollution [3].

Increasing evidence suggests that Zn supplements, even in modest doses, may have serious repercussions [38]. Intakes of Zn in the range of 100 to 300 mg/day may be prescribed by physicians as treatment for various medical problems, such as sickle cell anemia and celiac disease. However, prolonged therapy with such doses has been shown to cause severe Cu deficiency [4,38]. Zinc toxicity may also induce a loss of as much as 50% of Fe from the liver [3]. Both of these losses may result in anemia. Zinc intakes of 100 to 300 mg/day have also been reported to reduce the immune response, and affect blood lipids by causing increased LDL and decreased HDL levels [39]. Reports of alterations in serum lipoproteins have also been observed on lower doses of supplementary Zn (in the range of 50 and 75 mg/d) [38].

Although parenteral administration of Zn has been recommended at a level of 2.5 to 4 mg daily [26], much higher doses have been infused. Profuse sweating, blurred vision, and hypothermia have been reported after 5 daily doses of 10 mg each infused over 1 hour [2]. There have been a few reported cases of parenteral Zn poisoning. However, in the cases which have been reported in medical journals, most instances were due to errors in prescription [40].

COPPER

Biochemistry

Copper is an essential trace element which occurs in the adult human body at levels of approximately 75 to 150 mg [41]; two thirds are present in the liver and brain, with the remaining portion dispersed in variety of other tissues such as the heart, skeletal muscle, bone marrow, kidney, spleen and skeleton [42,43]. Serum Cu levels range from 70 to 155 μg/dL but are influenced by gender in healthy individuals [44]. Women have higher mean Cu levels than men, due to the stimulation of ceruloplasmin release by estrogens, and this situation is further exaggerated during pregnancy and oral contraceptive use [45].

Physiological roles

The primary function of Cu is its role in metalloenzymes, ie, cuproenzymes which catalyze oxido-reduction reactions. Important in humans are ceruloplasmin, ferroxidase II, Zn-Cu superoxide dismutase, lysyl oxidase, tyrosinase, monoamine oxidase and cytochrome-C-oxidase, among others. Cuproenzymes almost universally control the rate-limiting steps in their respective biochemical pathways. Therefore, based on the function of cuproenzymes, Cu is involved in erythro- and leukopoiesis, bone mineralization, connective tissue formation, oxidative phosphorylation, catecholamine metabolism, melanin formation, synthesis of thyroid hormones, antioxidant protection of the cell, and Fe turnover [41]. Copper is valuable with respect to normal Fe utilization because it is imperative for transferrin formation (ie, Cu required for ferroxidase action which oxidizes Fe for transport via transferrin), which explains a similar anemia (microcytic, hypochromic) in the setting of Fe or Cu deficiency. There is also evidence suggestive of a role for Cu in ascorbic acid metabolism, phospholipid metabolism and myelin formation, glucose homeostasis and in cellular immune defense [15].

Metabolism

In normal humans, 25 to 40% of the Cu ingested is absorbed [43]. Copper absorption is thought to take place mainly by simple diffusion in the duodenum [42]. In the mucosal cell it binds to a high molecular weight fraction and to metallothionein. Metallothionein acts as an intestinal block for Cu. The synthesis of metallothionein is induced by Zn, and therefore, at high Zn intakes, the increased amounts of metallothionein bind Cu making it unavailable for serosal transfer. This forms the basis for the interaction between Zn and Cu [46]. A number of other dietary factors affect the absorption of Cu. Copper absorption is enhanced by Cu deficiency and is depressed by ascorbic acid, Cd, and phytates in the diet [43]. It is likely that many endogenous chemicals, medications, and nutrients are in contact with Cu and affect its absorption.

On reaching the bloodstream, Cu becomes complexed with albumin and free amino acids and is transported to the liver and bone marrow. Copper in the liver is incorporated into ceruloplasmin and is released into the circulation or excreted into bile. Within the bone marrow, Cu is incorporated into erythrocuprine and released into the red cells [43]. Intravenous Cu is not accumulated by extrahepatic tissue until after its appearance in ceruloplasmin. Cells of the body appear to have membrane receptors for binding ceruloplasmin as well as mechanisms for reducing and disassociating the bound Cu atoms [47].

The major route of Cu excretion from the body is in the bile. Biliary excretion amounts to 0.5 to 1.3 mg of Cu per day [43]. Only 10 to 60 μg Cu/day are excreted daily in the urine. Small amounts are lost through hair, saliva, skin desquamation, menses, and sweat. Diarrhea increases Cu losses but not in proportion to the volume of diarrhea [43]. Excess corticosteroid use and trauma increase urinary Cu excretion [43]. The role that the various excretory pathways play in Cu homeostasis is not known.

Since Cu is such an ubiquitous element in plants and animals, it is widely distributed in foods. The richest sources include liver and shellfish. Other sources, in descending concentration, are nuts, high-protein cereals, dried fruits, poultry, fish, meats, legumes, root vegetables, leafy vegetables and fresh fruits [4]. Cow's milk is one of the poorest sources in our food supply [42].

An analysis of representative Canadian diets have indicated Cu intakes of 1.6 to 2.1 mg/day [4]. Until further studies are done in an attempt to establish adult Cu requirements, a suggested intake of 2 mg/day is considered to be safe and adequate [4]. Balance studies carried out on children aged 3 to 10 years suggest Cu requirements from 0.05 to 0.1 mg/kg/day [42]. As in the case for adults, insufficient data exist to establish firm recommendations.

The American Medical Association expert committee [26] has established suggested daily intravenous infusion levels for Cu. For adults they are 0.5 to 1.5 mg daily. This may be excessive given studies by Shike et al [48], showing a zero balance on 0.25 mg/day and Cu retention and accumulation on 1.05 mg daily. For children the suggested dosage is between 10 and 50 μg/kg/day [43]. This recommendation has also been questioned. Shulman [49] found positive Cu balances in children with persistent diarrhea as well as those who were postsurgical patients, while receiving the recommended parenteral Cu dosage. Allowances should be made, at least in infants, for exudative intestinal fluid losses. For low-birth-weight infants the 20μg/day recommendation achieves Cu balance. However in the premature low birthweight infant this amount has been shown to lead to a positive balance.

Extreme care should be used in prescribing parenteral Cu to patients with conditions predisposing to hepatic or whole-body Cu accumulation (eg, external biliary obstruction, primary biliary cirrhosis). Even in the case of normal hepatic metabolism, supplemental Cu has been shown to be retained [50]. Thus, in long-term TPN therapy, the chance of eventually producing total-body Cu overload, even with the recommended Cu intakes, is possible.

Deficiency

In general, the occurrence of Cu deficiency in humans is rare. It has been observed in premature infants [51], patients receiving Cu-free TPN [52], individuals receiving Zn therapy for sickle cell anemia [53], patients with chronic diarrhea, patients with malabsorption treated with high doses of Zn [27], and in women whose normal diet was supplemented with antacids [54]. Whether marginal Cu deficiency is more common has not been investigated.

In children the clinical manifestations of Cu deficiency are related to the decreased activities of the cuproenzymes. These include anemia, due to reduced ferroxidase, leading to impaired transport of Fe to erythropoietic sites; skeletal and vascular effects, due to decreased lysyl oxidase and amine oxidase activity which catalyzes the cross-linking in collagen and elastin; achromotrichia, due to impaired melanin synthesis, as a result of reduced tyrosinase activity; and central nervous system disturbances due to myelinization derangements, abnormal catecholamine concentrations associated with decreased dopamine ß-hydroxylase activity, and decreased activity of cytochrome-C oxidase [42]. Vascular aneurysms, growth retardation, hypercholesterolemia, and hypotonia have been reported in acquired Cu deficiencies in children [45].

Copper deficiency is virtually unknown in adults except those receiving chronic TPN. Copper deficiency was the first trace element deficiency associated with TPN [45,52] and Cu deficiency has since been documented in infants, adolescents and adults receiving TPN [45]. The most commonly reported features of TPN-induced Cu deficiency are hematological-microcytic anemia and neutropenia. Symptoms of Cu deficiency associated with long-term parenteral therapy have included diffuse osteoporosis, delayed bone age, edema of the limbs with pseudoparalysis, neurologic abnormalities, anemia, neutropenia and very low serum concentrations of Cu and ceruloplasmin [15]. All symptoms of Cu deficiency can be reversed with administration of Cu.

Menke's, or steely hair, syndrome is an inborn error of Cu metabolism. Patients present with the blood, bone, and cardiovascular abnormalities associated with acquired Cu deficiency, in addition to characteristic white hair with peculiar twisting, brittleness and lack of pigmentation and severe mental retardation [27]. It is thought that this disorder is caused in part by a defect in Cu absorption, with resulting Cu accumulation in the intestinal mucosa, low serum Cu concentration, and depletion of Cu at the cellular level [27]. The syndrome cannot be treated with oral or parenteral Cu and death usually occurs in early childhood.

Methods used to assess Cu status in humans include measurements of Cu concentrations in blood and its various components, hair, and the activities of certain enzymes. The hematological parameters are nonspecific for Cu deficiency, although the combination of neutropenia and a microcytic, hypochromic anemia with a low reticulocyte count in the presence of adequate Fe nutriture is extremely suggestive of Cu depletion.

Toxicity

Copper is very efficiently metabolized and thus toxicities rarely develop in humans. When they do arise, especially due to cases of acute poisoning, they can prove fatal, resulting in ulceration of the intestinal mucosa, hepatic cell necrosis, nausea, vomiting, diarrhea, hemolysis, hemoglobinuria and jaundice [55,56]. Several cases of accidental Cu toxicity in humans have been described, arising from the ingestion of Cu-sulfate, acidic drinks in prolonged contact with Cu containers, or drinking water with abnormally high Cu concentration (800 μg/L) [36]. High Cu intakes, strictly from the diet, pose no threat of causing Cu toxicity. However, massive introduction of Cu directly into the circulation has been shown to result in hemolytic anemia and hepatic necrosis, often with fatal outcome [45]. This would involve gram amounts of Cu, and a 100 to 1000-fold error in prescription would result in such an amount being delivered to a TPN patient. Liver accumulation and hepatocellular damage are possible long-term consequences of chronic administration of Cu supplements in excess of the biological requirements of patients [45].

Wilson's disease is an autosomal recessive disorder resulting in Cu accumulation and toxicity, primarily in the liver, brain and cornea. Clinical presentation may involve a hepatic, neurologic, or psychiatric picture. Normal individuals achieve Cu homeostasis by excreting excess or regulatory Cu in the bile, eventually to be lost in the feces. However, in those individuals with Wilson's disease there is some kind of metabolic block in this excretory pathway. Thus, the disease usually involves cirrhosis and lenticular degeneration, increased urinary Cu, decreased fecal Cu, aminoaciduria and low levels of ceruloplasmin [27]. The disease can be fatal if left untreated. Treatment of the disease involves the use of an anticopper agent, such as Zn, which acts by induction of intestinal cell metallothionein and blockade of intestinal Cu absorption. Other anticopper agents are penicillamine and trien, both of which are chelating agents causing increased excretion of Cu in the urine.

MANGANESE

Biochemistry

Manganese is considered an essential trace element for humans. The total amount of Mn contained in the human body is approximately 10 to 20 mg. The highest concentrations of the element are found in tissues rich in mitochondria, such as the liver, pancreas, and kidney. About 25% of the total body Mn content is present in the skeleton from which it is not readily mobilized [57]. Erythrocytes have been shown to account for approximately 66% of the Mn in whole blood, whereas the buffy coat (ie, platelets and leukocytes) accounts for approximately 30% [58]. Normal serum Mn reference ranges have been reported to be 0.43

to 0.76 ng/mL, and for whole blood 10 to 11 ng/mL [59].

Physiological roles

In addition to being present as an integral part of metalloenzymes, Mn functions biochemically as a cofactor which activates a number of enzymes. This second function is considered to be non-specific, since few enzymes show lowered activity in Mn deficiency. Manganese ions can activate many enzymes (eg, kinases, decarboxylases, transferases) which are also activated by Mg due to their chemical similarity [57]. Manganese is important in the homeostasis of the metabolic synthesis of proteins (eg, mucopolysaccharides and prothrombin), as well as carbohydrate and lipid (eg, activation of lipoprotein lipase, cholesterol, and sex hormone precursors) [15]. Manganese may be necessary for the action of vitamin K at the point in the reaction where the carbohydrate component of prothrombin is added to the prothrombin protein [43]. Manganese is a specific activator of the glycosyl transferases and is, therefore, required for normal glycosaminoglycan and glycoprotein synthesis (which are important components of connective tissue). There is growing evidence that intracellular, ionic Mn(II) plays a unique cofactor role in certain pathways in intermediary energy metabolism [60]. It is possible that greater efficiency in energy utilization might occur by maintaining normal Mn stores during TPN [60]. Manganese-containing metalloenzymes are found primarily in the mitochondria. Manganese is an integral component of arginase, pyruvate carboxylase, and mitochondrial superoxide dismutase [57]. The element is known to play a part in the normal function of the brain, with both Mn deficiency and toxicity affecting brain metabolism. Precise physiological functions for this element need to be further elucidated.

Metabolism

Manganese is absorbed throughout the length of the small intestine with absorption estimated to range from 3 to 8% [61]. However, absorption rates from 12 to 50% have also been reported [41]. The mechanism of absorption remains to be determined.

Generally, Mn absorption is not known to be affected by many dietary factors [62], though data on the bioavailability of dietary or parenteral Mn are scarce. However, lactose has been shown to increase Mn absorption in rats [63], and ascorbic acid and meat-containing diets have been shown to increase Mn bioavailability [4]. Divalent trace elements such as Fe and Co, and various dietary fibre sources, such as wheat bran and hemicellulose, decrease Mn bioavailability [4].

The majority of absorbed Mn is bound to an alpha-2-macroglobulin and is taken up by the liver. A portion of hepatic Mn is oxidized, bound to transferrin, and released into the circulation for uptake by other tissues [64]. Manganese is taken up by the mitochondria and more slowly by the nuclei [43]. Intravenously administered Mn possesses a slower rate of clearance from the body than orally ingested Mn. This may place the individual receiving intravenous Mn at risk of toxicity [65].

Manganese is largely excreted via the bile into the feces (99%) and to a lesser extent in intestinal secretions and pancreatic juice. Homeostatic regulation of Mn levels mainly occurs through excretion, rather than through regulation of absorption [43,66]. Urinary excretion of Mn is minimal, representing overflow losses and a higher fraction of total loss when intake is high.

Dietary Mn primarily comes from fruit and grain products. The mean Mn intake of adult Canadians is estimated to range from 3 to 3.8 mg/day [4]. The United States National Academy of Sciences has suggested an "estimated safe and adequate intake" of Mn for adults of 2.5 to 5.0 mg/day. Since no evidence of Mn deficiency exists in the Canadian or American population, present intakes are considered (but by no means are proven) adequate. Because of the lack of studies on Mn requirements, insufficient data exist to establish firm recommendations.

Although no case of Mn deficiency has ever been reported in patients receiving TPN, this element was among those considered indispensable for adequate nutritional maintenance during TPN. The American Medical Association expert committee has thus suggested a maintenance intravenous delivery of 0.15 to 0.8 mg/day Mn for stable adult TPN patients and 2 μg/kg/day for pediatric patients, with amounts adjusted downward for patients with biliary disease [26]. A recommendation for Mn in low birth-weight infants has been suggested to be 2 to 10 μg/kg/day [67].

Deficiency

Evidence for the occurrence of overt Mn deficiency in humans is largely lacking. There have only been two studies reporting Mn deficiency in humans [68,69]. In the first, an adult male on an inadvertently Mn-free purified diet designed to induce vitamin K deficiency developed symptoms thought to be associated with Mn deficiency. These included weight loss, fine skin rash, nausea and vomiting, reddening of hair colour, hypocholesterolemia and prolonged prothrombin time. Prolonged prothrombin time did not respond to vitamin K supplements until Mn was provided. Generalizations from this case to other clinical situations have been difficult. In a second prospective 39-day study, five of seven subjects fed a purified diet containing 0.11 mg of Mn per day developed a skin rash and hypocholesterolemia, symptoms similar to those seen in the study by Doisy [68]. There have been no reported cases of TPN-induced Mn deficiency. However, TPN has been associated with negative Mn balance when solutions are not supplemented with the element [60].

Even though Mn deficiency is extremely rare, its main features, seen in all species, include impaired growth, skeletal abnormalities, disturbed reproductive function, ataxia of the newborn and defects of lipid and carbohydrate metabolism [70]. The effect of Mn deficiency on the skeletal system can partially be explained in terms of its effects on mucopolysaccharide synthesis [70]. Animal studies have indicated a teratogenic effect of maternal Mn inadequacy [71]. There is the theoretical possibility that this could occur in a human pregnancy if the woman were deprived of Mn. Since TPN is now more frequently used in cases of pregnancy, the avoidance of such adverse consequences demands adequate Mn infusion to pregnant women receiving TPN.

Monitoring Mn nutritional status presents a number of problems. Functional changes with subclinical deficiency symptoms or early toxicity are not well defined. The determination of Mn in biological materials is not well developed and the relationship of circulating Mn to total-body stores is poorly understood. However, urine Mn is used in conjunction with serum Mn to evaluate possible deficiency or toxicity. Normal urinary Mn ranges are $<2.0\ \mu g/L$ but vary with laboratories. Levels may fall to $0.2\ \mu g/L$ (tenfold) in experimental deficiency [59].

Toxicity

Manganese toxicity in humans mainly results from environmental contamination. Clinical symptoms of Mn toxicity include nausea, vomiting, headache, neuropsychiatric abnormalities with disorientation, memory loss, hallucinations, anxiety, and compulsive laughing or crying [59,61]. In the more chronic form, Mn toxicity resembles Parkinson's disease [59,61]. Normalization of serum levels may not completely reverse the neurological damage.

Whole blood levels that have been associated with symptoms of Mn toxicity have ranged from 20 to 400 ng/mL [59]. Whole blood Mn levels have been shown to rise in individuals chronically exposed to the element, suggesting that iatrogenic overload may be able to be detected by measuring this index [60]. Urinary Mn levels ranging from 11.2 to 216.0 μg/L have been reported in factory workers exposed to Mn and experiencing Parkinsonian-like symptoms [59].

Toxicity caused by oral ingestion is extremely rare. No similar consequences are known with dietary Mn and are thus not to be expected from parenteral delivery of maintenance doses. However, since Mn is excreted in the bile, the adminstration of parenteral Mn to patients with cholestatic conditions may lead to the rise of serum Mn levels and hence, symptoms of toxicity.

SELENIUM

Biochemistry

Selenium is an essential element for humans. The total body content of Se varies relative to the amount present in the soil where individuals reside. In North America, the body content of Se is 13 to 20 mg, compared to levels of 3 to 6 mg in adults from New Zealand where there are low soil levels [72]. Tissue Se stores are negligible ($< 1\mu$g/g). The highest concentrations are found in the kidney, followed by the liver, muscle, and skin. Muscle contains almost half the total body Se content. Blood levels of Se vary greatly. Concentrations in whole blood range from 58 to 234μg/L whereas serum levels range from 46 to 143 μg/L [73]. Blood levels of Se in persons living in seleniferous areas may be ten times higher than those living in low Se regions.

Physiological roles

One of the established functions of Se in humans is as a component of glutathione peroxidase, an enzyme which assists in protecting cellular components against oxidative damage. The function of Se is, thus, related to that of vitamin E, and the interrelationship between the two is reflected in a sparing effect of each nutrient on the requirement of the other. Another selenoprotein, selenoprotein P, found in human plasma is thought to function as a transport protein for Se and as a defense against oxidative stress [72].

Selenium affects the metabolism and toxicity of a variety of drugs and chemicals, and protects against the toxicity of Ag, Cd, and Hg [74]. More recently Se has been found to be part of the enzyme that converts T_4 to the active thyroid hormone T_3. Selenium effects all components of the immune system and it is necessary for its optimum performance. In general, a Se deficiency appears to result in immune suppression [75]. Other physiological roles for Se have not yet been characterized.

Metabolism

Selenium is very efficiently absorbed, principally in the duodenum, by a nonregulated mechanism that is unaffected by Se nutriture. Tracer studies have shown that about 80% of dietary Se is absorbed [76]. Absorption is dependent on a number of variables. In particular, sulfurated amino acids can significantly increase absorption [15]. The utilization of orally and parenterally administered Se depends on the specific chemical form of Se. Oral and parenteral selenomethionine is retained better than sodium selenite [72].

Selenium is mainly consumed in the form of amino acids such as selenomethionine from plants and selenocysteine from animals. Following absorption, Se is bound to albumin and after being processed by red cells, circulates in association with beta-lipoprotein. It is then taken up by the tissues of the body. The selenomethionine released in digestion is used nonspecifically for the synthesis of tissue proteins. Humans also convert selenomethionine to selenocysteine, which is incorporated selectively into glutathione peroxidase [43].

Selenium is primarily excreted in the urine, but significant losses also occur through the feces. Less than 5% of the element is lost via the lungs and skin. The concentration of Se in the urine varies from 7 to 160 μg/L. Urinary Se losses are increased in catabolic states as well as during the delivery of parenterally administered sulfate [77].

Cereals are the most important source of Se in the Canadian diet [78]. However, cereals vary in their Se content, depending on the soil Se content in various geographical locations. Other important food sources of Se are seafoods, liver, kidney, and muscle meats; fruits and vegetables are generally low in Se. The chemical form, as well as the amount of Se in foods has nutritional implications since it has been shown that the bioavailability of the element in foods varies widely [79]. For example, Cu and vitamin C can reduce selenious acid to elemental Se, which cannot be used by the body. The chemical nature of Se in foods requires further investigation.

North American dietary surveys indicate that the average adult consumes between 100 to 200 μg/day of Se [4]. Based upon the results of oral balance studies which have shown that male adults require approximately 70 μg/day of Se to replace losses and maintain body stores, and because this level has neither been associated with Se deficiency or toxicity, the American Food and Nutrition Board has established that the recommended dietary allowances for Se for infants under one year of age, children one to ten years, and adults are 10 to 15, 20 to 30, and 40 to 55 μg/day, respectively [80]. Higher values of Se intake are recommended for pregnant and lactating women; 65μg/day and 75μg/day, respectively [80]. Canadian values have not been firmly established.

The American Medical Association expert panel did not include any recommendations for intravenous Se delivery during TPN. Intravenous dosages of 100 μg/day selenomethionine [81] and 120 μg/day Se in inorganic form have been used with success in adult TPN patients [77]. However, most stable patients on long-term TPN maintain normal blood Se values while receiving 40μg/day in their home TPN solutions [82]. Requirements may be greater in patients with high Se losses (eg, nasogastric aspirations, inflammatory bowel) [72].

There is little information regarding suitable intravenous Se dosages for infants. The American Society for Clinical Nutrition (Subcommittee on Pediatric Parenteral Nutrient Requirements) has recommended that preterm and term infants and children be given 2μg/kg/day up to a maximum of 30μg/day only in those pediatric patients receiving TPN for longer than 4 weeks [83].

Deficiency

Biochemical Se deficiency was determined to be prevalent in geographical areas with low soil Se content, such as China, New Zealand and Finland. Selenium prevents the development of Keshan disease, a juvenile cardiomyopathy caused by an extremely low Se intake. The disease is distinguished by congestive heart failure with moderate to severe enlargement of the heart and edema of the face. Keshan's disease has been virtually eradicated by Se supplementation. Inadequate Se nutrition has also been linked to Kashin-Beck disease, an endemic osteoarthritis with necrosis of the cartilage as the basic lesion [72].

The clinical manifestations of Se deficiency include muscle weakness, myalgia, myositis, increased red cell fragility, pancreatic degeneration, macrocytosis, and skin and hair depigmentation [84]. Similar to Keshan's disease, a potentially fatal cardiomyopathy can occur in long-term TPN patients receiving solutions unsupplemented with Se [85,86]. Attempts have been made to link low Se dietary intakes with increased incidence of cancer and cardiovascular diseases, but there is little reason to suspect that Se, at the levels found in most North American diets plays any role in these conditions. Persons most susceptible to a Se deficiency include premature infants, children undergoing growth, and individuals who primarily consume foods grown in low Se soils [72].

A number of reports have clearly illustrated that the use of TPN solutions that do not contain added Se result in decreased blood levels of Se and reduced glutathione peroxidase activity in adults, children, term, and preterm infants [87-91]. In addition, erythrocyte macrocytosis and pseudoalbinism have been reported in association with Se deficiency and long-term TPN [92]. Two cases have been reported of adults on home TPN who died of cardiomyopathies associated with severe Se deficiency [93,94]. Selenium supplementation of patients on TPN who complain of muscle weakness and/or painful myalgias are reported to reverse these symptoms.

Selenium status is most commonly assessed by measuring the Se content in blood. Cutoff points for serum Se concentrations associated with Se deficiency or toxicity, have not been defined in North America. Biologically active Se can also be estimated by measuring erythrocyte glutathione peroxidase activity, which is useful for detecting Se deficiency but does not reflect Se status in individuals with adequate Se stores [84]. Urine Se is used in conjunction with serum Se to assess Se nutrition or potential toxic exposure. Urinary Se levels $< 15 \mu g/L$ probably represent unusually low intake without necessarily representing illness [84].

Toxicity

Excessive intake of Se can be toxic. The margin between deficiency and toxicity is narrower for Se than for most other trace elements. Toxicity of Se due to long-term industrial exposure has been described [95]. In most cases of short term Se exposure, such as accidental ingestion, the majority of the Se is excreted via the kidneys without pathological consequences. However, the biochemical mechanisms by which Se exerts its toxic effects are not clear. Selenium toxicity generally involves individuals living in seleniferous areas. Intakes of 38000 μg/day Se occur in parts of China [96]. Selenium toxicity is characterized by chronic dermatitis, loss of hair and nails, excessive fatigue, dizziness, headaches, nausea, vomiting, pulmonary edema, circulatory collapse, and garlicky breath (due to the presence of dimethylselenide).

Serum Se levels >500 ng/mL and urine levels >500 μg/L, are associated with toxicity [84].

The amount of dietary Se necessary to produce toxicity remains to be determined. No signs of abnormality have been observed with Se intakes of 100 to 600μg/day, whereas 1500 μg/day have been found to be toxic [72]. In general, Se toxicity due to excessive dietary intake is not a significant health problem, with the exception of a few specific regions of the world. To date no cases of Se toxicity have been associated with the provision of TPN.

CHROMIUM

Biochemistry

The total amount of Cr contained in the human body has been approximated at 1.7 mg [97]. It is widely distributed, in low concentrations, throughout the body, and does not appear to be concentrated in any known tissue or organ. Circulating blood levels of Cr have been accepted to range upward from 0.038 μg/L [98].

Trivalent Cr (Cr^{+3}) is the most stable chemical form present in biological systems and is responsible for its nutritional activity [97]. There is evidence that Cr must be complexed with certain ligands to be fully functional [99]. Nicotinic acid and glutathione have been shown to be constituents of a Cr complex demonstrating superior in vitro activity [99]. This complex has been termed "Glucose Tolerance Factor". However, its exact chemical composition remains unknown.

Physiological roles

Trivalent Cr has been established as an essential nutrient for the maintenance of normal glucose metabolism in humans and animals [100]. The primary physiological function of Cr is to potentiate the action of insulin, thereby, reducing the amount of insulin required to control blood sugar and related processes. Chromium in the form of glucose tolerance factor is thought to facilitate the interaction of insulin with its receptor on the cell surfaces [101].

Another interaction is that of Cr with nucleic acids. Trivalent Cr bound to a specific high molecular weight ligand has been found to bind to nuclear chromosomes resulting in significant stimulation of RNA synthesis [99,101]. This suggests a regulatory role of the protein-bound Cr in nucleic acid synthesis.

Metabolism

Chromium can enter the body through the skin, digestive tract, and respiratory system. The metabolism of Cr is influenced by the route of entry, its oxidation level, and the nature of its ligands [97]. The main route by which trivalent Cr enters the body is through the digestive tract [97].

Chromium is rapidly absorbed in the intestinal mucosa with the jejunum appearing to be the most active portion for Cr absorption [97]. The absorption of dietary Cr is low and is a function of the daily intake. At a daily intake of 10μg, nearly 2% of the Cr is absorbed, while at intakes of 40μg absorption decreases to 0.5%. At intakes greater than 40μg, absorption remains constant at 0.4% [97]. The absorption of Cr is higher in women than in men, and this is thought to be related to the lower caloric content of the diet of women [97]. It has been proposed that Cr in the form of natural biologically active glucose tolerance factor complexes (eg, brewer's yeast), is better available for absorption than simple inorganic Cr salts [100]. However, it has recently been shown that inorganic Cr is equally well absorbed [97].

The mechanism responsible for the intestinal absorption of Cr has not been fully defined. However, results of experiments using a double perfusion technique, have shown that inorganic trivalent Cr is absorbed by a passive diffusion process and that under certain conditions (eg, in Zn and Fe deficiencies), Cr can be absorbed by binding to transport proteins [97].

Newly absorbed Cr circulates as free Cr^{+3}, bound to transferrin, or as complexes such as glucose tolerance factor [97]. At very high concentrations, Cr can bind nonspecifically to other plasma proteins, such as alpha- and beta-globulins and lipoproteins [97].

Circulating trivalent Cr is taken up by the tissues, with its bodily distribution dependent upon species, age, and chemical form [97]. Excretion of absorbed Cr occurs primarily through the urine (approximately 0.5 - 1.5 μg/day based on an average dietary intake of 50 - 200 μg/day), and in small quantities in the hair, sweat, and bile [97]. Since so little Cr is absorbed, the feces are the major route of elimination after oral ingestion. Urinary losses are enhanced by glucose loading, insulin secretion, diabetes [102], and exercise [15]. Urinary excretion provides a good indication of the recent intake of Cr, but not in predicting its body status [97,99]. Total Cr losses in healthy adults have been reported to be approximately 10μg daily, and this is increased to about 19.2 μg per day in diabetics [102].

In the diet, Cr is present in the inorganic form and as organic complexes. Trivalent Cr combined with glutathione and nicotinic acid (glucose tolerance factor) is present in the diet in brewer's yeast, in pig liver and kidneys, or synthesized from Cr^{+3} in the body [97]. It is necessary that inorganic Cr be transformed to a biologically active form in order to have a physiological role. The Cr content of individual food varies widely and depends upon the Cr introduced from external sources during growing, processing, preparation, fortification, and handling of foods [103]. Most dairy products are low in Cr, as are most meats, fish, and poultry. Grain products, fruits and vegetables vary widely in their Cr content, with some foods providing > 20 μg/serving [103].

The U.S. National Research Council Food and Nutrition Board has suggested a range of safe and adequate intake for Cr of 50-200 μg/day [80]. Typical Cr intakes in Canada have ranged from 56 to 282 μg/day [4]. There is evidence that well-balanced diets may contain levels of dietary Cr below the suggested intake [103]. Thus, marginal Cr deficiency may exist in several well-developed countries.

Current guidelines for the addition of Cr to parenteral nutrition solutions are based on recommendations made by the Nutrition Advisory Group of the Department of Foods and Nutrition, American Medical Association. Daily intravenous Cr delivery is recommended at the following levels: pediatric patients 0.14-0.2 μg/kg, stable adult patients 10-15 μg, and stable adult with intestinal losses 20μg [26].

Deficiency

The most common symptom of Cr deficiency is an impaired glucose tolerance, as evidenced by hyperglycemia, glucosuria, and glucose intolerance requiring insulin therapy [99]. Other symptoms include high free fatty acid levels and abnormal nitrogen metabolism [104]. Neuropathy [104] and encephalopathy [105] have been reported but these symptoms may be later manifestations of the deficiency [106]. Other documented features of Cr deficiency in laboratory animals include decreased growth rates, increased mortality in young animals, decreased longevity in mature animals, corneal opacities, elevated serum cholesterol, and increased incidence of aortic atherosclerosis [107]. Chromium deficiency has been indisputably proven in protein-calorie malnutrition [108]. However, signs and symptoms of marginal Cr deficiency in countries possessing a rich supply of foods also appear widespread because of the suboptimal intake of dietary Cr, and because of the high consumption of simple sugars that increase urinary Cr losses [15]. Three cases of Cr deficiency have been reported in individuals receiving TPN without added Cr. Chromium deficiency in patients undergoing TPN may be due to continuous glucose loading, resulting in a higher urinary excretion of Cr, and in turn increased requirements [102].

There are yet no specific sensitive tests available to determine the nutritional Cr status of an individual [99]. Presently, the best way to diagnose a Cr deficiency is to demonstrate an abnormal glucose tolerance that responds to Cr supplementation [102].

A hypothesis proposed over twenty years ago by Schroeder [109] that Cr deficiency represented a significant risk for cardiovascular disease has received renewed interest. Abraham et al [110] observed a reversal of established atherosclerosis in the aorta of cholesterol-fed rabbits receiving daily Cr injections. Human controlled studies with Cr compounds have produced mixed results. Two studies with hypercholesterolemic individuals found a significant increase in the HDL cholesterol level of Cr supplemented groups, with no improvement in total cholesterol level [111,112]. However, Wang et al [113] demonstrated a significant reduction in total and LDL cholesterol and Press et al [114] showed a significant reduction of total and LDL cholesterol and an increase in HDL cholesterol. Because of the numerous nutritional influences on lipid metabolism it is realistic to assume that only certain cases are related to a compromised Cr status. Further controlled human studies are necessary to confirm an effect of Cr on lipid metabolism.

Toxicity

Chromium as pure metal produces no toxic effects. However, hexavalent Cr has long been recognized as a toxic substance. Hexavalent Cr compounds appear to be 10-100 times more toxic than trivalent Cr when both are administered orally [108]. The National Institute for Occupational Health and Safety Registry data on the acute oral toxicity of trivalent Cr compounds range from 1900 to 3300 mg/kg body weight. By comparison, the LD_{50} for Na-dichromate, a hexavalent compound, is reported to be 50-150 mg/kg body weight [108]. The main toxic effects after contact, inhalation, or ingestion of hexavalent Cr compounds are the following: dermatitis, allergic and eczematous skin reactions, skin and mucous membrane ulcerations, perforation of the nasal septum, allergic asthmatic reactions, bronchial carcinomas, gastroenteritis, hepatocellular deficiency, and renal oligo anuric deficiency [115]. Ascorbic acid directly influences the toxic effects of Cr; it converts hexavalent Cr to trivalent Cr, thereby, reducing its toxic effects [116]. A very large number of epidemiological and isolated case studies have concluded that the trivalent compounds do not appear to be carcinogenic [115]. The hexavalent compounds of moderate solubility are carcinogenic and mutagenic, and the highly soluble compounds are very cytotoxic and mutagenic [108]. Mutagenicity and related studies have sufficiently shown that hexavalent Cr is genetically active. In contrast, trivalent Cr compounds are inactive in most test systems, except in systems where they can interact directly with DNA [108]. Both oxidation states, when injected at high levels parenterally in animals are teratogenic, with the hexavalent form accumulating in the embryos to a much greater extent than the trivalent [100].

BORON

Biochemistry

Boron is distributed throughout the tissues and organs of the body in small concentrations, mostly between 0.005 and 0.6 μg/g fresh weight [117]. Small amounts of B in this range, in increasing order, have been found in brain, testes, muscle, liver, kidney, lung, and lymph nodes [117]. The B content of ashed human bones ranges from 16 to 138μg/g [117]. Reported blood levels of B have remained consistent since the 1950's, with a mean concentration in serum of 0.18 to 0.21 μg/g [117].

Physiological roles

Although there is evidence that B may be essential for animals, an essential function has not been confirmed in humans. There are experimental findings to support the hypothesis that B has an essential function that somehow regulates parathormone activity, and therefore, indirectly influences the metabolism of Ca, P, Mg, and cholecalciferol [117]. However, the data need to be clarified by further experimentation. It has been statisitcally shown that the B content in human teeth is associated with a low caries incidence. However, high levels of orally administered B have been shown to increase dental caries or antagonize the cariostatic effect of F [117]. There is evidence to indicate that B is involved in cell membrane metabolism. Also, B compounds, amine cyanoboranes and amine carboxyboranes (B analogues of alpha-amino acids), have been shown to block induced arthritis [117]. Tablets containing Na-borate have been praised as a remedy for arthritis [117]. Boron may also have a role in some enzymatic reactions in humans because it has been shown to affect the activity of a number of enzymes in vitro and in plants. Other functions of B remain unknown.

Metabolism

Boron in food is rapidly absorbed and excreted mainly via the urine. In one of the few known human balance studies involving B, >90% of a 352 mg oral dose of B as boric acid was recovered during a period of one week from the urine [117].

Intake of B from Western diets has been reported to be 2000 to 4000 μg/day [117]. Foods of plant origin provide major sources of B [4]. The highest levels of B have been reported in nuts, fruit, leafy vegetables, legumes and honey [117]. Wine, cider, and beer are also high in B. It is apparent that the daily intake of B by humans can vary widely depending on the proportions of various foods in the diet. Since dietary deficiency of B has not been reported in humans under normal conditions, the above intake is presumed to be adequate.

Deficiency

Explicitly stating the signs of B deficiency in animals is difficult because of the varied nature of the symptoms and of the diets used to induce deficiency. It has been suggested that human B requirements may be so low that frank deficiency may not be observed in humans. Nevertheless, a number of reports have appeared with findings that B deprivation affected variables associated with Ca or bone metabolism in both animals and humans, especially when they were exposed to stressors altering macromineral metabolism. In general, when experimental animals are fed nutritionally optimal diets, there is little response to dietary B. Responses to dietary B have been the most marked when an experimental animal has had to respond to a nutritionally or metabolically stressful situation which adversely altered hormonal or cellular membrane status (eg, Ca, K, Mg, and/or cholecalciferol deficiency). In humans, B may also be of nutritional significance under certain nutritional or metabolically stressful circumstances (eg, low dietary Mg intake, an age associated with increased loss of Ca from bone or the body).

Recently, an inadequate dietary intake of B was suggested to be an important factor in the etiology of osteoporosis [118]. In nutritional studies performed with human subjects, B supplementation (3 mg/day) tended to change several of the variables associated with Ca metabolism (eg, calcitonin and 25-hydroxycholecalciferol) in the men and women (not on estrogen) in a way that made them appear like women on estrogen therapy. This suggested that B and estrogen may have similar beneficial effects on Ca metabolism and in the prevention of bone loss which occurs in postmenopausal women and older men [119].

Electroencephalogram (EEG), sensory-motor, and cognitive performance data were obtained from human B depletion-repletion studies. During low B intake, subjects demonstrated impaired performance in various tapping, pursuit, counting, search and encoding tasks. EEG's showed altered performance of a number of brain areas. Together the findings suggest B deprivation depressed mental alertness [119].

Toxicity

No cases of toxicity due to B intakes from normal diets have been reported. There is, therefore, thought to be a considerable margin of safety with respect to the oral intake of this trace element. Toxicity signs are thought to generally occur only after dietary B concentration exceeds 100μg/g. In animals fed diets with high concentrations of B, toxicity signs included coarse hair growth, edema, depressed hemoglobin and hematocrit, reduced growth, and gonadotoxic effects (eg, testicular degeneration and cessation of spermatogenesis, and impaired ovarian development). In humans the signs of acute B toxicity include nausea, vomiting, diarrhea, dermatitis, and lethargy [117]. Additionally, high B intakes induce riboflavinuria [117]. Animal studies have indicated a marked increase in the B levels of tissues, particularly the brain, when large amounts of boric acid are ingested [117]. When sheep were raised on soil with a high B content, the B contents of the organs were elevated with the greatest increases occurring in the spleen, kidney and brain.

ALUMINUM

Biochemistry

It is not known whether or not Al belongs to the group of trace elements considered essential for humans. The primary concern with this element is in regard to its toxicity. Aluminum is one of the most common elements in the environment and, thus, exposure to it is rather inevitable. Aluminum can be detected in all human organs. The total body Al content of an average man has been estimated to be 30 mg [120]. Normal Al levels for various human biological samples are as follows: serum, plasma or whole blood $<5\mu g/L$; bone 1.5 to 13.3 $\mu g/g$; and brain 0.74 to 3.56 $\mu g/g$ dry weight [121].

Metabolism

Relatively little is known about Al absorption. However, it appears that the lungs, skin, and gastrointestinal tract provide excellent barriers to Al under conditions of normal environmental exposure. Absorption is thought to occur in the acidic environment of the stomach and proximal duodenum, since high acidity has been shown to increase the solubility of Al [121,122]. The absorption of Al may involve an active process, in which it may compete with Ca [123]. The availability of ingested Al is influenced by such factors as nutritional deficiencies of divalent cations and the F content of food and water supplies. Aluminum decreases F and Fe absorption from the gastrointestinal tract and conversely F and Fe decrease Al absorption [121,124]. Aluminum also decreases P and Ca absorption [124].

Investigations have indicated a direct absorption of inhaled Al in the lungs. A few research groups suggest a secondary intestinal absorption of inhaled Al. It is assumed that Al particles are transported by way of the absorption epithelium of the respiratory tracts in the direction of the pharynx and swallowed [121].

Aluminum circulates in the blood in ultrafilterable and protein-bound fractions, with both albumin and transferrin aiding this function [4]. In plasma, approximately 80 to 95% of Al is bound to proteins [121]. The unbound plasma Al has access to intracellular compartments, thus allowing for tissue accumulation with Al loading [125].

In healthy individuals absorbed Al is largely eliminated by the kidney. The precise mechanism for Al handling by the kidney remains unknown. After saturation of plasma protein binding, unbound Al is likely excreted in the urine [125]. Urine Al levels have been reported to be less than $14\mu g$/day for normal persons [121]. In cases of renal failure absorbed Al is retained and can severely alter the body Al burden with resulting toxicity. However, when very large oral loads of Al are administered to individuals with normal renal function some of this Al is also retained [124]. Biliary excretion of Al may also play an important role in elimination [126]. Most ingested Al is not absorbed and is excreted as Al-phosphate in the feces [121].

In general, it appears that at low levels, the body is well equipped to handle Al. This is achieved through a combination of low gastrointestinal absorption, plasma binding proteins and renal excretion of the free fraction, thus averting accumulation of all but minimal amounts of Al [4].

Aluminum is naturally present in trace quantities in water and most foods. Further increases in the Al content of drinking water may arise since Al-sulfate is usually added during the purification process. Municipal water can contain up to 1000μg/L Al, thus making it a potentially important source of Al intake [124]. Higher amounts of Al are found in plant foods and some fish and relatively lower amounts are contained in most meat and dairy products. The concentrations of Al in various foods generally range from 0.02 to 5 mg/kg wet weight [127]. In addition to natural sources, Al may be added during the processing, preparation, and storage of foods. Considering all dietary sources, it has been estimated that the average adult ingests between 1 to 30 mg Al/day [127], with a representative amount generally being quoted as 20 mg/day [4,128]. Less than 1% of this amount is absorbed [121].

The quantities of Al in foods are small compared to the amounts that can be ingested by way of pharmaceutical products. With the ingestion of various drugs, total daily Al consumption can easily escalate. A number of nonprescription drugs such as antacids, buffered aspirin, and antidiarrheals can contain high amounts of Al depending on their formulation. Potential daily Al doses arising from the nonabusive use of antacids and buffered aspirin may reach 5000 mg or 730 mg, respectively [125]. Sucralfate, used in the treatment of peptic ulcer disease, contains 828 mg Al in the recommended therapeutic dosage of 1g/day. The Al in this medication has been shown to be absorbed [129]. It must be noted that there is generally insufficient information about the forms of Al in many foods and pharmaceuticals and, hence, their bioavailability and toxicity. However, the low body burden of average healthy individuals would suggest that any Al absorbed is largely, if not entirely, eliminated from the body [124].

Toxicity

Aluminum is toxic to the gastrointestinal tract. Symptoms of toxicity include general fatigue, anorexia, vomiting and weight loss [4]. Constipation is observed with the increased intake of Al (eg, in antacids) since the element directly inhibits smooth muscle contractility [124].

Some symptoms of Al toxicity are caused by direct interaction of Al with biomolecules such as nucleic acids, lipids, peptides, proteins, carboxylic acids and membranes, in addition to interaction with essential minerals [128]. For example, several investigators have observed that large oral doses of Al interfere with P absorption and lower tissue P level. The bone pain and fractures observed in patients who have used large doses of Al-containing antacids for years has been related to a P-depletion syndrome [128].

Aluminum toxicity has been documented as a result of direct parenteral exposure (eg, in TPN or hemodialysis) where the gastrointestinal tract has been bypassed, or in association with therapeutic use of pharmaceuticals. Some chronic renal failure patients on long-term dialysis exhibit osteomalacia, brain encephalopathy associated with dementia and/or dialysis induced microcytic anemia [130-133]. These disorders are all due to high Al intakes from Al-containing phosphate binding gels and the use of Al-containing water for dialysis. Additionally, treatment with the anti-ulcer medication sucralfate has been associated with Al toxicity. In patients with chronic renal insufficiency (CRF) who received therapeutic doses of sucralfate (1g/day) for 3 weeks, significant elevation in serum and urinary Al were reported [129]. Serum values did not return to baseline until 1 week after discontinuation of the drug, and urinary values remained elevated for 2 weeks after discontinuation of the drug. Such studies have led to restricted use of sucralfate in CRF patients due to its potential for Al toxicity. Patients receiving TPN have also been found to accumulate Al in their tissues from Al-containing solutions (eg, Ca-gluconate) [134]. Reduced bone formation and osteomalacia have been described in adult and infant patients with normal or near normal renal function receiving long-term TPN [134-137]. The mechanism of the neurotoxic effect of Al has not been clarified.

Individuals with hyperparathyroidism can also develop Al neurotoxicity because Al absorption is enhanced in the presence of elevated circulating parathyroid hormone. Parathyroid hormone leads to the preferential deposition of Al in the brain and bone [138].

There has been interest in the toxicity of Al as a potential pathogenic factor in a number of human diseases. Aluminum has been implicated in diseases of the brain, blood, bone and lungs. However, recent public health concerns surrounding Al exposure have targeted the possible role Al may play in the etiology and pathogenesis of Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinsonism-dementia as seen in Guam [139,140]. Increased Al concentrations have been detected in several brain regions of patients with Alzheimer's disease, with Al being concentrated in the neurofibrillary tangle-bearing neurons [141]. Various epidemiological reports have indicated that the risk of developing Alzheimer's disease is correlated with a high Al content in drinking water [140,142]. Presently, Al is considered to be involved in the pathogenesis of the disease rather than the etiology. There is currently no evidence to verify that these diseases are directly induced by Al toxicity [121].

To evaluate the true Al body burden, tissue and bone have been suggested as the specimens of choice [121]. However, since these are difficult to obtain, blood Al levels have been analyzed to determine an individual's Al load. Most methods use serum or plasma as an indicator specimen. Urine samples have also been used for biological monitoring of occupationally exposed individuals.

BARIUM

Biochemistry

A Ba level of approximately 20 mg has been found in the average-sized person, 93% of which is localized in the bone [143]. Trace amounts of Ba are found in various tissues such as the aorta, brain, heart, kidney, spleen, pancreas, and lung [144]. Tissue Ba levels vary in subjects from different countries. The mean Ba level in 103 samples of whole human blood was reported to be $0.1 \pm 0.06\ \mu g/g$ [117]. The total Ba content of humans, thus, depends upon the geographical location of the individual.

Physiological role

Barium has not been conclusively shown to perform any essential function in living organisms. The metabolism of Ba is similar to Ca. Barium can replace Ca in many physiological processes, and it has been shown to affect nerve and muscle activity [144].

Metabolism

The absorption of ingested Ba is difficult to assess because of the number of factors that affect it. For example, the presence of sulfate in food results in the precipitation of Ba-sulfate. Additionally, absorption of Ba from the gastrointestinal tract depends upon the individual's age and the solubility of the compound. Only about 6% [117] of an ingested quantity of Ba is believed to be absorbed by adults; however, absorption may be higher in children [143]. Inhaled Ba compounds are absorbed through the lungs or directly from the nasal membrane into the bloodstream. Poorly soluble Ba compounds collect in the lungs and are slowly removed by ciliary action.

Absorbed Ba enters the bloodstream with uptake occurring rapidly in the salivary and adrenal glands, heart, kidney, mucosal tissue, and blood vessels, and is finally deposited in bone. The mechanisms by which Ba is deposited in body tissues are not well known. Barium metabolism is similar to that of Ca and Sr, and like these elements, Ba accumulates in bone.

Barium is excreted in both the feces and urine, with elimination varying according to the route of adminstration and the solubility of the compound. The elimination of ingested Ba by humans occurs principally in the feces. In healthy humans in a state of Ba equilibrium (where virtually all of Ba intake occurs by mouth), approximately 91% of the total output was found in the feces, 6% in sweat, and 3% in the urine [144].

The diet provides the main route of Ba exposure. Based on data from the United States, the dietary intake of Ba ranges from 600 to 900 μg/day [143]. Barium is usually associated with Ca and Sr in the food chain. Limited information exists with respect to the Ba level in human foods. Brazil nuts appear to be an unusually high source of Ba with reported concentrations being 1500 to 3000 μg/g. Tea and cocoa have been reported to contain a high Ba content compared to a number of other beverages. Bran flakes and instant cream of wheat contain high levels compared to a number of cereals. Eggs have been reported to contain 0.76 mg/100 g and swiss cheese 0.22 mg/100 g. Fruits, fruit juice, and vegetables contain relatively low Ba levels. In one report, all meats showed concentrations of 0.04mg/100g Ba or less [144].

Recent American studies have reported Ba levels in drinking water to range from 1 to 20 μg/L. Based on this range and assuming a daily consumption of 2 litres drinking-water, the daily intake of Ba from water would be 2 to 40 μg. Certain regions of the United States have reported Ba concentrations of up to 10000 μg/L [143]. Canadian water supplies have been reported to range from 5 to 600 μg/L [144].

Toxicity

Barium's effects in living systems depend upon the solubility of the salt in question. Some Ba salts are quite soluble in water (eg, acetate, nitrate and chloride) while others are essentially insoluble (eg, arsenate, carbonate, oxalate, sulfate, phosphate). Soluble salts are extremely toxic to living cells. Most Ba salts are insoluble in water and these have a high density which makes them opaque to X-rays [145]. The use of Ba-sulfate as a contrast medium for radiography depends entirely upon its insoluble nature.

Barium doses as low as 0.2 to 0.5 g (3 to 7 mg/kg body weight) resulting from the ingestion of Ba-chloride or carbonate have been found to lead to toxic effects in humans [143]. In untreated cases, doses of 3 to 5 g (40 to 70 mg/kg body weight) were lethal. Clinical signs of Ba poisoning include: acute gastroenteritis, loss of deep reflexes with the onset of muscular paralysis, progressive muscular paralysis, hypokalemia, delayed kidney damage, vasoconstriction, and irregular heartbeat. Death may occur from cardiac or respiratory failure. Ingestion of high levels of Ba exert profound effects on all muscles, especially the heart. Muscular paralysis appears to be associated with severe hypokalemia. In most reported cases of Ba poisoning, quick recovery occurred after treatment with infused K salts and/or oral administration of Na-sulfate [143].

Even though it is considered to be essentially nontoxic, insoluble Ba-sulfate used for contrast media in radiography has revealed long-term retention of Ba and granuloma of the transverse colon [145].

The findings of early epidemiological studies relating exposure to low levels of Ba with cardiovascular disease and mortality proved indefinite. No increase in the incidence of high blood pressure, stroke, or heart and kidney disease was observed in a population exposed to high concentrations of Ba in drinking-water when compared to control groups [143].

An increased incidence of hypertension was reported among workers exposed to Ba, compared with non-exposed workers. A study group consisting of Ba-exposed workers and people residing near a landfill site containing Ba was found to have an increased prevalence of musculoskeletal symptoms, gastrointestinal surgery, skin problems, and respiratory symptoms [144].

There is a lack of conclusive evidence that Ba compounds, with the exception of Ba-chromate, are carcinogenic in humans, nor is there any conclusive evidence that Ba produces reproductive, embryotoxic, or teratogenic effects in humans [143]. Barium, at concentrations found in the environment, does not pose any significant risk for the general population. However, for those under conditions of high Ba exposure (eg, high water content, occupational exposure) the potential for adverse health effects should be considered [143].

VANADIUM

Biochemistry

Tissue levels of V are generally low, in the ng/g weight range. Reported values for human tissue range from 0.55 to 7.5 ng/g and appear (in increasing order of concentration) in fat and muscle, heart, lung, kidney, thyroid, and liver [146]. The liver is one of the principle organs that retains V.

The level of V in human body fluids such as blood and urine remains controversial. Data from a number of studies has indicated a normal serum V level of 0.061 ± 0.05 ng/mL [146]. It is clearly evident that if V has a physiological role, that it is required in ultratrace amounts.

Physiological Roles

Vanadium is an active element in vitro and pharmacologically, therefore, numerous biochemical and physiologic functions for it have been suggested. There is some evidence that V might have a role in regulation of (Na,K)-ATPase, phosphoryl transfer enzymes, adenylate cyclase, and protein kinases. There has also been discussion of the potential role of V in hormone, glucose, lipid, bone, and tooth metabolism, and as an enzyme cofactor in the form of vanadyl [147]. However, no specific biochemical function for V has been identified in humans.

Early studies with rats and chickens indicated that V is important in growth and development [146]. Additionally, animal studies have indicated that after acute and long-term administration of V compounds, a decrease in plasma cholesterol occurs. In in vitro studies, V has been shown to inhibit cholesterol biosynthesis in the liver. Vanadium compounds have, thus, been used therapeutically in humans for the treatment of disease. For example V has been given orally (21 to 30 mgV/day) as diammonium oxytartarovanadate, for six weeks, in a study on its cholesterol-lowering effects. Vanadium compounds have also been applied to teeth to study their effectiveness in preventing dental caries.

The finding that some haloperoxidases in lower life forms require V suggest a similar need in higher animals. The most familiar haloperoxidase in animals is thyroid peroxidase. Vanadium deprivation has been shown to affect the response of thyroid peroxidase activity in altering dietary I. Many actions of V can also be explained by its having a role similar to, or enhancing that of, growth factors. Vanadium mimics growth factors such as epidermal growth factor and insulin. Since 1985, numerous studies have examined the mechanism through which V mimics the action of insulin and prevents signs of diabetes associated with streptozotocin adminstration to rats. A mixture of vanadate and H_2O_2 was found to produce a synergistic effect that amplified insulin-like growth factor II binding to rat adipocytes and activated insulin receptor kinase. The synergistic insulin-like effect was apparently caused by peroxide(s) of vanadate, which has been named pervanadate [147]. Although definitive studies of the physiological properties and necessity of V have yet to be performed, there is increasing evidence suggesting that it is an essential trace element.

Metabolism

Vanadium can be absorbed through the respiratory and gastrointestinal tracts, although the gastrointestinal route is the most studied. Vanadium occurs in the diet or as a pharmacological agent, mainly as vanadyl or vanadate. Vanadate is transformed to vanadyl in the stomach, which is primarily the absorbed form. Most studies indicate that relatively little (0.1 to 1%) of ingested V is absorbed [148]. However, other dietary components likely affect its absorption. Absorbed V is mainly transported in the plasma. The main route of excretion of absorbed V is the urine with lesser amounts lost in the feces, probably via the bile [146]. Because of the low level of gastrointestinal absorption, however, most ingested V is eliminated unabsorbed with the feces. Urinary V concentrations tend to be higher in those most heavily exposed to the element.

No V requirements for humans have been established because of weak evidence regarding its essentiality. However, if requirements do exist they are extremely low and likely able to be met by the levels occurring naturally in food, water, and air [146]. Although the daily intake of V may vary widely, is generally thought to be in the range of 10 to 30 μg/ day [146,148]. Sources of V are bread, some grains and nuts, vegetable oils, fish, meat and a few vegetables. The amounts vary from less than 0.1 ng/g in peas, beets and carrots, to 52 ng/g in radishes. Liver, fish, and meat contain up to 10 ng/g [146].

Deficiency

No deficiency syndrome for V has been characterized, though, the nutritional studies performed in animals to date appear to demonstrate its importance in growth and development.

Anke and co-workers used goats in V-deprivation studies and found that goats fed a diet insufficient in V exhibited a greater abortion rate, produced less milk during lactation, possessed depressed serum glucose, skeletal deformations, increased thyroid weight, and decreased growth when compared to control animals [150]. Vanadium is required for the activity of nitrogenase and haloperoxidase in lower life forms. Since thyroid function requires the action of thyroid peroxidase (a haloperoxidase) and thyroid function is essential in growth and development, it can be speculated that the deprivation effects of V are mediated via the thyroid gland. Additionally, the insulin-like properties of V, especially its effects on mitogenesis, suggest that this element plays a role in growth and development.

Toxicity

Vanadium compounds possess varying degrees of toxicity, acting mainly as irritants to the conjunctivae and respiratory tract. Controversy exists as to the effects of industrial exposure on other systems of the body. Symptoms and signs of poisoning are pallor, greenish-black discolouration of the tongue, sporadic cough, conjunctivitis, difficulty breathing and chest pain, bronchitis, and tremors of the fingers and arms [146]. There have also been reports of cardiovascular effects with chronic exposure to high levels of V, such as arrhythmia, bradycardia and coronary spasm; hepatic effects such as enlarged liver and bilirubinemia; and hematological effects such as anemia [148]. Vanadium poisoning can be diagnosed on the basis of a history of exposure, the clinical picture as described above, and the presence of elevated V levels in the blood and urine. The summary toxicity statement (THR) of V-pentoxide equals: high via oral inhalation, intratracheally, subcutaneously, and intravenously [146]. Vanadium is not very toxic to humans when ingested due to poor absorption from the gastrointestinal tract. However, when introduced intravenously in a soluble form, the lethal dose of V-pentoxide is 0.42mg V_2O_5/kg body weight [148].

The reported effects of V compounds on the human organism require further research. Available data do not imply any risk of carcinogenic effects, however, the data cannot be considered conclusive. There is weak evidence of possible mutagenic effects of V compounds in animal studies, and scanty evidence concerning spermato- and gonadotoxic effects. Available data suggest that V may be embryotoxic and gonadotoxic. However, results indicating the induction of teratogenicity in animals and humans require further study [148]. There is no evidence that the general population is at risk of either V deficiency or overexposure [149].

TITANIUM

Biochemistry

Few measurements of the Ti content of body tissues have been made and reference levels for Ti in biological fluids have not been determined. The greatest concentrations of Ti have commonly been found in the lungs, probably as a result of dust inhalation, followed by the kidney and liver. The total body Ti burden in the soft tissues of the reference human is approximately 9.0 mg, 2.4 mg of which is in the lung [151]. Titanium values from fresh tissue have been reported to range from 0.2 to 10 μg/g. In level of increasing concentration, Ti is present in bone, brain, kidney cortex, liver, and lung tissue [117,151]. Serum levels of Ti have been reported as 90μg/L [151]. However, it must be noted that there is little comparative data to comment on the accuracy of these results.

Physiological role

There is no evidence that Ti is an essential element for humans or animals. Little information exists regarding the biological effects of Ti.

Metabolism

The gastrointestinal absorption of dietary Ti is very low, approximately 1 to 3% of intake [152]. Most of the ingested quantity is excreted in the urine and feces. In humans, Ti is thought to be excreted in urine at an approximate average rate of 10 μg/L [152]. Excretion by other routes is unknown.

Titanium crosses the blood-brain barrier and is also transported through the placenta into the fetus [152]. Experiments with rats suggest that Ti may be taken up by the lungs from the blood [152].

Most human ingestion of Ti occurs as TiO_2 in food. Titanium dioxide is used as a food additive where whiteness is desired (eg, in dairy products and bread flour) and is used as a colour stabilizer in toothpastes [151]. Although there is some variation with diet depending on the Ti content of foods grown in different areas and the quantity of processed foods consumed, the average daily oral intake is in the range of 100 to 1000 μg/day [151]. This accounts for approximately 99% of the exposure in the general population. Titanium in drinking water is generally low, about 2 μg/L, and is considered a minor source [151]. Very little is known about the Ti content of human foods. Large variations in the concentration of Ti in different types of foods have been demonstrated. Whole grains, some vegetables and fruits, and common fish meat have been found to contain little or no detectable Ti, while higher levels ranging from 1.76 to 2.42 mg/kg wet weight have been found in milled grains, butter, corn oil, and lettuce [152]. Higher concentrations of Ti can occur in food crops in localized areas as a result of soil contamination.

Toxicity

Few data exist on the systemic effects of Ti and its compounds. Titanium is used quite safely in large quantities as a food additive and is exceptionally nontoxic [151]. Titanium and Ti-containing alloys are widely used as biocompatible prosthetic devices. Titanium in implants and prostheses are extremely well tolerated by osseous and soft tissues [152]. Iatrogenic exposure from prosthetic devices has been considered, but analyses of body fluids adequate to assess the extent of leeching have not been achieved [151]. Titanium dioxide and other Ti-compounds have been used in various dermatological and cosmetic formulations without any known adverse effects [152]. The inclination of Ti to form biologically inert TiO_2 is fortunate, given the abundance of the element in the environment and the difficulties associated with its accurate measurement.

Available data on the occurrence of Ti and Ti compounds in the environment, as well as data on toxicity, indicate that the current level of exposure of the general population does not present a health risk [152]. Dose-effect and dose-response relationships have not been established for any of the effects of various Ti compounds. According to animal studies, inhalation of Ti compounds may have fibrogenic effects. There is yet no evidence of Ti being carcinogenic or teratogenic in humans. No quantitative assessment of human health risk from exposure to Ti compounds in occupational or non-occupational environmental situations can be made at this time [152].

STRONTIUM

Biochemistry

In general, the tissue distribution of Sr mimics that of Ca. Major sites of retention of both elements include the skeleton and teeth, with the aorta falling a distant third [117]. The Sr content of bone has received much interest because of the possible problem of Sr^{90} retention from radioactive fallout. The total Sr content of the standard reference man has been reported to be 323 mg, with about 99% of the body burden being deposited in bone [117,153]. Strontium occurs in the enamel and dentin of teeth in a wide range of concentrations [117]. Strontium has been reported to be contained in a variety of human organs (in increasing concentration): muscle, brain, kidney, liver, aorta, and lung [117]. There is evidence that the Sr levels in human bones and enamel, as well as in lungs and aorta, increase with age and vary among geographical regions [117]. The Sr concentration in blood and bone, is influenced by the dietary intake of Sr, Ca, and phosphate. Plasma Sr concentrations have been reported to range from 10 to 217 μg/L [153]. This large variation may be attributable to the different analytical techniques used for Sr measurement.

Physiological role

There is no convincing evidence that Sr is essential for living organisms. However, in 1949 it was reported that the omission of Sr from diets fed to rats and guinea pigs resulted in growth depression, poorly calcified bones and teeth, and higher incidence of dental caries [117]. This report has neither been corroborated or negated. Other studies have since indicated that some of the Ca requirement of growing chicks could be spared by Sr. Further support for this hypothesis came from a study which found that the inhibition of beef brain cortex adenylate cyclase activity in vitro by the chelating agent EGTA, though reversed completely by Ca^{+2}, was partially reversed by Sr^{+2} [117]. Strontium may, thus, be able to substitute for Ca in some enzyme systems. In biological membranes, Sr has the unique ability to replace Ca even at sites highly specific for Ca [153]. Of the Sr compounds existing, those containing phosphate are of main interest because from a chemical perspective they are closely related to Ca-phosphate compounds found in calcified tissues.

Dental surveys suggest that low prevalence of caries has a curvilinear relation to the Sr concentrations in drinking water. However, the association of Sr and F together in water appears to be necessary for a maximum reduction in caries [153]. The Sr-fluorapatite that forms has greater resistance to dissolution than Ca-hydroxyapatite.

Metabolism

The metabolism of Sr has mainly been investigated in relation to the metabolism of Ca because both not only share chemical characteristics, but also have similar involvement in biological processes. Despite the large amount of research performed, it is difficult to summarize the available data about Sr metabolism because they are presented in such diverse manners (eg, ppm ash, ppm wet weight, mg Sr/g Ca, Sr/Ca ratio). However, wherever there is a metabolically controlled passage of ions across a membrane (eg, gastrointestinal absorption, renal excretion, lactation, and placental transfer) Ca tends to be transported more effectively than Sr [117]. Nevertheless, physiological and nutritional variables that affect Sr metabolism are comparable to those that affect Ca metabolism and usually operate in the same direction. For example, parathyroid hormone accelerates the resorption of bone Sr, as it does Ca [117].

The gastrointestinal tract is the main route of Sr entry into the body. The fraction absorbed via the intestine (5 to 25%) is relatively low compared to the fraction absorbed via the lungs (85%) [117]. Both animal and human studies have shown that the intestinal absorption of Sr and Ca are analogous, which led to the hypothesis that these elements share a common carrier system in the intestinal wall. However, data suggest that, in contrast to Ca, Sr is absorbed entirely via passive diffusion [153]. Intestinal Sr absorption is negatively influenced by aging, chelating agents, food in general, and high dietary contents of Ca and/or phosphate [153]. Increases in dietary Ca intakes from low to normal reduces Sr retention, and supplementation with Ca and phosphorus is more effective in reducing Sr retention than Ca alone [117].

Strontium is transferred from blood to the interstitial fluid to a similar extent as Ca, but intracellular penetration appears to be limited [153]. Strontium is poorly retained by humans. In adults net retention is essentially zero, ie, a steady state exists [117]. The little that is retained is deposited preferentially in the bones and teeth.

Strontium is excreted in the urine, and to a lesser extent, in feces, milk, and sweat [153]. The normal Sr value for human urine has been reported to be 0.4 mg/L [117]. Strontium excreted in the feces originates from nonabsorbed, orally ingested Sr and Sr that is actively excreted by the intestine, the latter being directly related to the blood concentration. In human milk, an excretion of 0.14 to 0.34 mg Sr/ g Ca has been reported [153]. The sweat glands account for only a small portion of Sr loss.

The Sr content of human diets and the daily intake of the element vary according to the kinds of foods consumed and the geographical area where they are grown. Strontium occurs naturally in food and beverages. In general, foods of plant origin are richer in Sr than are animal products, except where the latter include bone [117]. Cereals, grains, and seafood contain up to 25 mg/g. Amounts over 100 mg/g have been found in Brazil nuts, cinnamon, and some kinds of fish flour made of whole fish including bones [153]. Like Ca, milk and milk products can contribute a large percentage of Sr intake; values between 11 and 32% of the total dietary intake have been reported [117]. The daily Sr intake from food is no more than a few milligrams.

In some geographical areas, the drinking water can contribute a substantial proportion of total Sr intake. In municipal water supplies in the United States, Sr levels have been found to range from 0.002 to 1.2 μg/L, depending on the source and hardness of the water [153].

Toxicity

Limited information is available on the toxicity of stable Sr, since most toxicity research has focused on the effects of radiation of radioactive Sr. In general, Sr appears to have a low order of toxicity. A wide safety margin exists between dietary levels of stable Sr likely to be ingested from ordinary food and water supplies, and those that induce toxic effects [117]. However, it must be noted that toxic levels confirmed in feeding studies strongly depend on the composition of the diet, especially the content of Ca and phosphate [153]. For example, feeding 6.0 mg Sr as carbonate per gram of diet to chicks depressed growth slightly at 1.0% dietary Ca, and severely depressed growth at 0.72% dietary Ca [117]. In 1922, it was shown that high dietary Sr could produce a syndrome known as "strontium rickets" in experimental animals, in which the skeleton fails to mineralize even in the presence of sufficient vitamin D [117]. Strontium may interfere with vitamin D metabolism, perhaps by lowering or blocking the ability of kidney enzymes to convert 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol.

COBALT

Biochemistry

The total body content of Co in a 70 kg adult has been estimated to be 1.1 mg, with 85% incorporated into cobalamines or combined with low molecular weight proteins [154], and 15% existing in the skeleton. The total amount of vitamin B_{12} in the body is 2 to 5 mg (ie, 0.09 to 0.22 mg of Co; the vitamin containing 4.34% of Co) [154]. Cobalt does not appear to accumulate in any target organ. However, the highest levels are found in the liver and kidney [154]. Serum or plasma levels have been reported at 0.327 μg/L. The values in whole blood range from 0.5 to 3.9 μg/L [154].

Physiological role

Cobalt is an unique trace element for humans since it can only reveal its essential properties if provided directly as its biologically active form, cobalamin, or vitamin B_{12}. Inorganic Co salts are, therefore, not considered essential micronutrients for humans.

Metabolism

There are two main routes of absorption of Co: inhalation and ingestion. The absorption of inhaled Co is approximately 30% and occurs quickly. The absorption of ingested Co is about 25% depending on the chemical form and nutritional factors such as the amount of Fe, amino acids, and proteins present in the meal [154]. Absorption of Co is similar to that of Fe, with which Co shares the absorptive pathways in the duodenal mucosa and proximal small intestine. Cobalt and Fe mutually compete, each inhibiting the absorption of the other [154].

The gastrointestinal absorption of vitamin B_{12} depends upon the presence of intrinsic factor which is formed in the stomach. In its presence about 70% of vitamin B_{12} can be absorbed while in its absence only 2% is assimilated [154].

The excretion of Co occurs in two phases. During the rapid phase, which ensues within a few days after ingestion, 80 to 90% of the Co is eliminated. In the second slow phase, with a biological half-life of 2 to 15 years, the remaining 10% is eliminated. Urine is thought to be the predominant route of Co excretion, but biliary excretion also seems to be an important determinant of fecal excretion according to studies with rats [155]. The mean value of Co in human urine has been reported to be 0.4 μg/L [154].

The only known human nutritional requirement for Co is that amount necessary for the structure of vitamin B_{12} [41]. The daily intake of Co by humans has been reported to be approximately 5 to 45 μg [154]. In Canada the recommended nutrient intake for B_{12} adults is 1 μg (0.04 μg of Co) [4].

Plants generally contain low amounts of Co but they are the main sources for animals. Cobalt is present in green vegetables at a range of 0.2 to 0.6 mg/kg dry weight. The content of animal foods ranges between 0.05 and 0.25 mg/kg dry weight, liver and kidney being the richest sources [154]. Animals are able to synthesize vitamin B_{12}, which is the primary source of Co in animal foods. However, only a portion of the Co obtained from animal foods is present as cobalamines. Higher levels of Co are present in certain seafoods. The Co content of drinking water is usually low (0.1 to 5 μg/L) [154].

Deficiency

A dietary deficiency of elemental Co (ie, not in cobalamin form) has not been described in humans. However, a deficiency of vitamin B_{12} leads to megaloblastic anemia and neurological disorders. This is often attributable to the absence of the intrinsic factor.

Toxicity

There have been no cases of Co toxicity due to intake from the normal diet. However, a high intake of Co from beer was implicated in severe cardiac failure in heavy beer drinkers in Canada and the United States [4]. The toxic dietary level (μg/g dry weight) of Co for animals is at least 25 times higher than the reported levels in the human diet. Doses of 3 mg/kg/day may be tolerated by sheep during several weeks without severe damage. There is, therefore, a relatively large margin of safety [4]. However, the ingestion or inhalation of large doses may lead to pathological disorders. For example, there has been some suggestion that excess Co interferes with I metabolism in rats and may lead to the development of goitres [41].

In acute cases of toxicity the main clinical manifestations are pulmonary edema, allergy, nausea, vomiting and hemorrhage. Cases of chronic toxicity have reported pulmonary syndrome, skin syndrome, allergy, gastrointestinal irritations, nausea, cardiomyopathy, hematological disorders, and thyroid lesions. Additionally, mutagenic, teratogenic, and carcinogenic actions have been observed, but must be explored in further detail before any concrete statements can be made [154].

ARSENIC

Biochemistry

The total body content of As has been estimated to be 0.31 to 4.0 mg [156]. The mean As content of most human tissues has been reported to be between 10 to 60 μg As/kg on a wet weight basis, but in most unexposed persons the levels are below this range [156]. The skin, hair, and nails have been shown to be consistently richer in As than other tissues. The level of As in human blood varies widely among individuals. Values for fresh blood in unexposed individuals range from 0.5 to 32.0 μg/L As [156]. Large differences in the As exposure via air, water, and dietary sources account for the wide range of concentrations described.

Physiological role

No proof has been found for the essentiality of As to humans. However, growth experiments with plants and animals point to the possibility that As is a necessary nutrient. The essentiality of As in growing, pregnant, and lactating goats and minipigs and their offspring have been systematically evaluated since 1973. The physiological role of As may be one that affects the conversion of methionine to its metabolites taurine, labile methyl, and the polyamines. In addition, it may be involved in the methylation of biomolecules (eg, histones) [147]. Safe and adequate intakes for As have not been defined.

Metabolism

The main route of As uptake is orally, in addition to inhalation of contaminated air (eg, during spraying of insecticides). Most As compounds are absorbed from 80 to 100% by the gastrointestinal tract [156]. The rate of absorption depends on the valence, kind, and physical form of the As compound. The absorption of As by the lungs is approximately 50 to 80% [156].

Arsenic is bound in serum to protein (alpha$_1$-globulin) and in red blood cells to the globin part of hemoglobin. Arsenic leaves the intravascular space within 24 hours and is incorporated into different tissues at varying rates. It is stored first in the liver, kidney, spleen, and lung [156]. The creatine sulfhydryl groups of the skin retain up to 30% of the absorbed As, and parts of this are later located in the hair and nails. Four weeks after ingestion, As is localized in bone substituting for phosphate [156].

The chemical form of As, as well as the species of animal appear to influence the metabolism of As to a large extent. Lower life forms undergo chemical reactions (eg, reductive biomethylation of As) in order to reduce As toxicity, and allow its processing by the organism. Often, the intermediates of As detoxification are toxic and cannot be allowed to accumulate. Humans likewise, must detoxify some As compounds. Compounds of As that enter the gastrointestinal tract are subjected to the action of bacteria and enzymes, and after absorption must pass through the liver before reaching the general circulation. This process alters the chemical form, especially of inorganic As compounds. The detoxification mechanisms of As in humans are methylation to monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) and excretion by the kidneys. Methylated compounds are less toxic and more rapidly excreted. Such in vivo methylation of As may occur enzymatically in the liver.

Organic forms of As tend to be quickly absorbed and rapidly excreted by the body. For example, humans have been shown to excrete 69 to 85% of the As in the form of arsenobetaine, the form found in seafoods, in the urine within 4 to 5 days of intake [157]. After poisoning, the main part of absorbed As is eliminated in urine 48 hours after overexposure. Within 5 to 7 days 60 to 70% is eliminated by the kidneys, approximately 6% by the feces, and traces in sweat and desquamated skin cells [156].

Urinary As excretion increases with elevated As intakes, and the total urinary As excretion provides a useful index of exposure. Most As, >95%, is excreted via the urine. The renal rate of As excretion depends upon the form. Humans not exposed to As and without ingestion of As-containing food usually excrete As as DMAA (80 to 90%) and only low amounts of inorganic As (10%) and MMAA (10%) [156]. During biological monitoring of humans, not the total As but rather the amounts and ratios of the different As metabolites are proof of As contamination. Normal urine levels of As vary between 1 and 80 μg/L; generally they are lower than 10 μg/L [156].

Excretion of As via the bile is influenced by the route of intake and chemical form. It is very low after oral intake, but increases to > 10% of the dose after intravenous injection in rats [157].

Most human foods contain <0.3 μg/g As and rarely exceed 1 μg/g on a dry basis [157]. Arsenic has been reported in (in order of increasing concentration) milk, meat, cereals, vegetables, fruits, and meat [157]. Seafood, especially crustaceans, contain relatively high concentrations of As, often as much as 100 mg/kg. However, almost all of this As is in the form of organoarsenic compounds (eg, arsenobetaine) which are nontoxic and are not metabolized to toxic forms in humans. Such forms are rapidly excreted via the kidneys. Studies of municipal water supplies found As concentrations of less than 0.01 mg/L in the United States [156]. Concentrations higher than this amount generally possess geological origins. Certain homeopathic medicines also contain considerable amounts of As. Depending on the therapeutic instructions the daily intake could be between 0.1 and 5 mg [156].

The total amount of As ingested daily by humans is influenced by the amount of seafood consumed. The daily As intake from Western diets has been reported as 20 to 100 μg/day [4]. Since a dietary deficiency of this trace element has not been reported in humans under normal conditions, this range of intake is presumed to be adequate.

Deficiency

Results of induced As deficiency in goats and rats have revealed depressed growth and abnormal reproduction characterized by impaired fertility and elevated perinatal mortality [157]. Histological changes induced by As deficiency mainly affected the mitochondrial membranes of the heart and skeletal muscle.

A number of biochemical changes due to As deficiency have been described in all species of animal examined, but the biological importance of the changes remain unknown. Depressed hematocrit and hemoglobin content of blood in growing As-deficient chicks was reported [157]. A deficiency of As was shown to cause changes in the mineral composition of the organs of goats and pigs, in their newborns, and in their milk. A reduced ash content of the skeleton (of goats), increased Cu concentration of the organs, and increased Mn content of the organs of the offspring and the milk was also reported in As deficient animals. In As-deficient goats a reduced triglyceride content was registered, which might indicate that As plays a role related to lipid phosphorus in biological systems [157]. The lower total microsomal liver protein found in the As-deficient animals may point to a more general role of As affecting amino acid metabolism or protein synthesis [157].

Toxicity

Target organs of As toxicity are the cardiovascular system, gastrointestinal tract, epithelial tissues, skin, kidney, and nervous system.

Metallic As, metal arsenides, and compounds with sulfur are not poisonous. Arsenobetaine, the main component of the so-called fish As in seafood, is also nontoxic. However, the white powder arsenious oxide, "arsenic", is one of the most well-known poisons in history. Doses of 0.01 to 0.05 g produce toxic symptoms. The lethal dose is 0.12 g, more probably 0.3 g; however recoveries from higher doses have been reported [156]. All soluble compounds of As are also toxic, such as As (III), and As (V) halides, arsenic pentoxide, salts of the arsenious (III-arsenite) and arsenic (V-arsenate) acid. Organic compounds of As are also poisonous. In general, organic compounds are less toxic than inorganic, and insoluble less toxic than soluble compounds.

Acute and chronic As intoxications have become rare in Western countries. The symptoms of nonfatal acute As poisoning in humans by the oral route include: nausea, vomiting, diarrhea, burning of the mouth and throat, and severe abdominal pains. Chronic exposure to smaller toxic doses of As are mostly insidious and nonspecific. Without a known history of contamination it is almost impossible to associate many of the symptoms with As. Symptoms include weakness, prostration, muscular aching with few gastrointestinal symptoms, and hearing loss in children. Skin and mucosal changes usually occur together with peripheral neuropathy and linear pigmentations of the fingernails. Headache, drowsiness, confusion, and convulsions occur in both acute and chronic cases of As toxicity [157].

The biochemical basis for such disturbances is likely inhibition of a wide range of enzyme systems by As. Arsenic(III) is the active arsenic form and combines with the sulfhydryl of many enzymes (eg, pyruvate dehydrogenase, DNA polymerase). Arsenic(V) substitutes for phosphorus, disrupting oxidative phosphorylation. The inhibition of cellular oxidative processes results in capillary injury and tissue hypoxia followed by vasodilation, transudation of fluid, and decreased cardiac output due to hypovolemia [156]. Arsenic is also seen as an inducer of genes involved in proliferation, recombination, amplification, and activation of viruses. This characterizes As as an indirect carcinogen [156].

The maximum long-term As intake compatible with health and general well-being in humans cannot be provided with any precision because of variations in individual susceptibility and because the chemical form of As significantly affects its toxicity [157].

It is still not certain whether or not As is a carcinogen for humans, since, apart from As, there are other carcinogenic heavy metals in the environment of examined populations. The length of exposure to As is of importance. Skin cancer has occurred in association with exposure to inorganic As compounds in a variety of populations (eg, workers engaged in the manufacture of pesticides). Lung cancer has been observed to be associated with inhalation exposure to As in Cu smelters, and in workers in pesticide plants [157]. Further investigation is necessary in order to clarify the risk of cancer due to As exposure in humans.

The total daily urinary As excretion and the levels of As in hair and nails possess considerable value in the diagnosis of As poisoning. Hair As values of $>3\mu g/g$ indicate possible poisoning [157]. Precise diagnostic criteria of potentially harmful As intakes or definitions of "safe" long term dietary As intakes are not available.

A large quantity of ingested As may be eliminated by gastric lavage, vomiting, and diarrhea. As toxicity is treated with the chelating agent dimercaprol, or British anti-Lewisite (BAL). In cases of high As doses and anuria, BAL therapy is combined with hemodialysis [156].

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CHAPTER 2

TRACE ELEMENT ANALYSIS

Analysis of Trace Elements Using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

This chapter discusses the theory behind ICP-MS technology, and specifically, the analysis of TPN components and solutions with the Perkin Elmer Sciex Elan 5000A ICP-MS System (Perkin Elmer, Toronto, ON). Secondly, it describes the necessary quality controls undertaken during sample preparation and analyses.

The most widely used clinical techniques for the analysis of trace elements are, flame atomic absorption spectrometry (FAAS), graphite furnace AAS (GFAAS), and inductively coupled plasma atomic emission spectrometry (ICP AES). However, the number of applications of inductively coupled plasma-mass spectrometry (ICP-MS) appearing in the scientific literature is profoundly increasing. Important areas of application include geological analysis, environmental analysis (including the analysis of water samples), analysis of metals and high purity materials, analysis of biological and clinical samples, and analysis of food samples.

Components of the ICP-MS

ICP-MS is an analytical technique to determine elements using mass spectrometry of positively charged ions generated by an inductively coupled plasma. Within the mass spectrometer the beam of gaseous ions are sorted according to their mass-to-charge ratios m/e (m = mass, e = number of electric charges lost or gained during ionization). The signals created are proportional to the relative abundance of each ionic species present: the mass-to-charge ratios of the ions allows a qualitative interpretation, while the measured ion intensities allows a quantitative analysis [1].

The Perkin Elmer Sciex Elan 5000A ICP-MS instrument employed for this research basically consists of an ion source, a mass analyzer, and a detector (Figure 2-1).

Ion source. In this instrument, an inductively coupled plasma (ICP) Ar torch serves as the ion source. The Ar plasma (described as a hot ionized gas) has a gas kinetic temperature of 6000 to 14000°C. Analytes introduced into this plasma are rapidly desolvated, vaporized, atomized, and ionized [2]. The ICP efficiently ionizes the elements to singly charged positive ions (M^+) so that the plasma can be used as an ion source for the mass spectrometer.

Mass analyzer. In ICP-MS analysis, the population of ions is then analyzed according to atomic properties, ie, mass-to-charge-ratio, with a mass spectrometer. The ideal mass analyzer allows the separation of ions with a small difference in mass (high resolution) and passes a large number of ions so that high sensitivity is achieved (high transmission). The ICP-MS instrument used for this project employs a quadrupole analyzer which transmits ions of the selected mass-to-charge ratio. This technique allows multielement determinations with extremely low detection limits [3].

Detector. The ICP-MS instrument is equipped with a channel electron multiplier (CEM) which is a continuous dynode electron multiplier of high gain. It is a curved narrow glass tube in the shape of a funnel and coated internally with a secondary electron emitting material (the continuous dynode). When a positive ion falls into the funnel, secondary electrons are emitted and accelerated along the tube. By collisions with the tube more electrons are formed, resulting in an avalanche of electrons [3]. The electronic pulse produced from the detected ions is sent to measurement electronics where the signal is converted to quantitative data output.

Other spectrometric analytical techniques

Flame atomic absorption spectrometry. FAAS is a single-element technique ideal when few elements must be determined in a large number of samples. Although interferences do occur, most are well identified and usually easily controlled. Sample sizes must be greater than 1 mL and detection limits for most elements range from 10 to 1000 μg/L. In addition, refractory elements such as B, Al, Ba, Ta, and Zr cannot be determined with good sensitivity because the flame temperatures are not high enough to atomize a large fraction of these analytes [4].

Graphite furnace atomic absorption spectrometry. The major advantages of GFAAS are that its detection limits (0.01 to 10 μg/L) are 10 to 100 times better than for FAAS or for ICP AES, and sample sizes can be as low as 10 to 20 μL. However, GFAAS also carries the disadvantages that refractory elements are difficult to determine, it is essentially a single-element technique, and analytical times are slow (ie, 3 to 6 minutes per sample). The chemical and physical matrix interferences which occur with this method require considerable awareness on the part of the analyst. Often, much method development may be required to control interferences [4].

Inductively coupled plasma atomic emission spectrometry. ICP AES is the clear competitor to ICP-MS in terms of simultaneous multielemental capability. ICP AES features high sensitivity for most elements, a broad linear dynamic range (10^6 in most cases), reasonable precision (0.5% when an internal standard is used), limited and increasingly understood matrix interferences, and relatively rapid analyses (approximately 10 to 60s per sample, with 15 to 30 elements per sample being determined). However, it has a major disadvantage of having relatively high detection limits (1 to 100 μg/L) [5].

Characteristics of ICP-MS

ICP-MS in its ideal embodiment retains most of the attributes of the ICP that are found in ICP AES but adds a number of strengths unique to mass spectrometry [5]. The analytical benefits of ICP-MS include: rapid multielemental analysis, relative spectral simplicity, rapid quantitative analysis, low detection limits, extremely broad linear dynamic working range, and isotopic analysis capabilities. ICP-MS possesses the ability to determine most elements of the periodic table (both in qualitative and quantitative modes), ie, from atomic mass 3 (Li) to 338 (U) with only Ar and He not being determinable [6]. Usually relatively simple spectra are seen, with each element having one peak for each isotope. There are generally few isotopic overlaps. A number of elements have detection limits in the range of 0.001 to 0.1 μg/L, rivalling or surpassing those of GFAAS [2]. Such detection limits can be obtained simultaneously for a large number of elements in each sample. Detection limits are typically 10 to 100-fold lower than ICP-AES and far exceed that offered by FAAS. A comparison of the detection limits of various analytical methods is presented in Figure 2-2. The linear dynamic range of calibration is about six orders of magnitude, comparable to ICP-AES. Present day instrumentation achieves a signal stability in the order of 0.1 to 0.5% RSD (relative standard deviation), essentially the same as that found in ICP AES. However, for this level of reproducibility an internal standard is routinely employed [5]. The precision of ICP-MS is reported to be 0.5 to 3% with accuracy depending upon the quality of the standards, presence of interferences, extent of contamination, as well as other factors [3].

Trace element analyses are performed with the Perkin Elmer Sciex Elan 5000A ICP-MS by two different methods. The "Total Quant" mode typically measures approximately 90 elements with the total mass range being measured per sample. Relatively short sampling times are used per mass, which sacrifices precision and detection limits for speed. This is a semi-quantitative method. During this type of analysis a standard containing four to ten elements is used to cover the atomic mass range being scanned. Such a setting is valuable for deriving the elemental composition of a unknown sample.

The second mode is used for "Quantitative" analysis. During this procedure, 10 to 30 elements are measured per sample with one selected mass being measured per element. Sampling times are relatively long (2 to 5 minutes) for each element to ensure adequate precision and detection limits. There is high accuracy in well characterized samples. In this procedure each element is specifically standardized. "Quant" analysis requires some prior knowledge of the sample matrix in order to plan for potential interferences, choose appropriate mass values, and make necessary corrections.

Interferences occurring during ICP-MS analysis

Despite the general spectral simplicity observed with ICP-MS, corrections are required for major interferences which include 1) isobaric interferences (mass overlaps occurring when two or more elements have the same mass number) 2) molecular interferences (where molecular ions are formed when one or more atoms react with an ion) such as polyatomic species and doubly charged ions, and 3) a variety of matrix interferences.

Isobaric interferences. Elemental isobaric overlaps occur when two isotopes of different elements have the same mass-to-charge ratio within the resolution of the mass discriminator. ICP-MS instrumentation employs correction software to overcome isobaric interferences; the element of interest can be selected to be measured at a certain mass number and a correction applied based on the known natural abundances of the isotopes. Fortunately, in most cases, another isotope of the analyte can be chosen that is free from isobaric interferences. Such a strategy is unavailable for monoisotopic elements like Mn^{55} and Co^{59}. The TPN components and complete solution samples analyzed in this study were essentially free of any isobaric overlaps due to careful isotopic mass selections by the analyst. The only element with a potential isobaric overlap was Zn^{64} with an isotope of Ni^{64}. Since there was no Ni present in our samples to provide this interference, Zn^{64} was chosen for determination of Zn concentration. However, a correction factor for this known isobaric interference was automatically applied by the instrument.

Molecular interferences. Molecular interferences are caused by the plasma gas Ar together with traces of air and water; by oxides of elements; by molecular species formed by major plasma components and major matrix species of the sample; and by atomic and molecular species formed as the product of reactions between the plasma components and the surfaces with which they come into contact. When the aforementioned polyatomic ions are produced from major elements in the sample they may interfere with the determination of trace elements having the same mass. If polyatomic interferences are significant in amount, the apparent concentration of trace element will greatly exceed the true concentration.

Because of the fact that Ar^{40} is often the heaviest nuclide present in high concentration, and most polyatomic ions are made up of only two components (sometimes with hydrogen as a third component), important background peaks are only seen for elements with mass number <82 [7]. The solvent has a particularly marked effect on the background spectra observed in ICP-MS. Water (which was the solvent of the solutions analyzed in this study) yields simple background spectra. Other elements present in the sample may also yield ions of the forms of MO^+, MOH^+ and possibly MH (oxide, hydroxide, and hydride). To overcome such interferences the spectrum is always "stripped" using an appropriate matrix matched blank. To minimize the detection of interfering molecular oxide species, the sampling cone of the instrument is positioned near the centre of the plasma, in that area containing purer forms of the produced ions. As the plasma cools further away from the centre of the plasma, oxide formation occurs. Sampling ahead of this point minimizes the amount of oxides likely to be detected.

Additionally, polyatomic Ar species are observed. For example, $^{80}[Ar_2]^+$ interferes with Se^{80}, the main isotope of Se [3]. Such coincidences can severely limit the detection limits that can be achieved in practice. However, this can often be overcome by choosing another isotope, eg, Se^{82}. In other cases, eg, for V and As, there exist no suitable alternatives [3].

Prior to any analyses, the composition of the TPN solution was studied. Any potentially interfering elements, and molecular combinations possessing similar masses to the elements selected for measurement were established. In this way correction factors for the potential molecular interferences could be applied. For example, our samples contained substantial levels of Cl. Therefore, for "Quant" analysis, a potential molecular interference for V^{51} with $Cl^{35}O^{16}$ was recognized and a correction factor was applied to the scan. Table 2-1 contains a list of selected ions and their respective correction equations used for quantitative analyses of TPN components and complete solutions.

Chromium was discovered to be a difficult trace element to measure in the TPN matrix. The molecule $Ar^{40}C^{12}$ interferes with the major isotope of Cr, Cr^{52}. Unfortunately, Cr^{53} also has a number of molecular interferences, including $Ar^{40}C^{13}$ and $Cl^{37}O^{16}$. Interferences caused by the extremely high C (in dextrose and Travasol) and Cl (in KCl and NaCl) content of the complete TPN solution, even when diluted, made the accurate analysis of Cr in the complete TPN solution futile. Reported Cr values were incredibly high due to the interferences. The individual inorganic component solutions did not interfere with Cr analysis, in which Cr^{52} was analyzed with a correction factor for the molecular interference with $Ar^{40}C^{12}$. Samples of TPN solution, dextrose, Travasol, and inorganic component solutions were sent to the Quebec Centre for Toxicology for measurement of Cr content via GFAAS. Results indicated a very low Cr content in the dextrose solution and Travasol solutions (<2.0 μg/L), and confirmed our results with the inorganic samples. A correction factor based on these results was attempted for the analyses of TPN solutions with minimal success. However, it was decided that Cr in the complete TPN solution could not be reliably corrected for and analyzed with the standard equipment presently used to operate the ICP-MS at the time of analyses. In Chapter 3, values for Cr in Lot 1 components of dextrose and Travasol are reported from GFAAS analysis, while Lots 2 and 3 of the two solutions are based upon ICP-MS analyses with the applied correction factors. Additionally, Cr values were unable to be confidently reported in complete TPN solutions analyzed via ICP-MS in Chapter 5.

Doubly charged ions occur for some elements with low ionization potentials, eg, Sr and Ba. If too much energy is imparted to atoms during ionization in the plasma, greater than one electron may be stripped off leading to the formation of doubly charged ions. Because the mass spectrometric detection system discriminates on the basis of mass-to-charge ratio, an ion of mass M and charge 1+ will be indistinguishable from an ion of mass 2M and charge 2+. To minimize the occurrence of doubly charged ions, the temperature conditions of the plasma were strictly controlled.

Matrix effects. A potential limitation of ICP-MS is the occurrence of matrix effects, ie, matrix-induced changes of the intensity of the signal. The importance of matrix effects depends upon the element to be analyzed, the matrix, and the operating conditions [3]. Matrix effects are associated with the sample introduction system, and therefore, must be controlled at this level. The control of sample uptake and nebulization, and thus the amount of sample entering the plasma, is maintained at a constant flow rate with a peristaltic pump. This ensures against fluctuations and maintains the consistency of the sample entering the plasma. The Ar gas flow rate (ie, plasma flow rate) is also controlled to maintain a consistent amount of sample entering the plasma and a consistent amount of ionization. To minimize matrix effects, component and complete TPN solution samples were matrix matched with all of the standards and blanks in a 0.5% nitric acid solution.

In most instruments, solutions are rarely analyzed that contain more than 1% w/w total dissolved solids because this reduces instrument sensitivity due to matrix effects [6]. To correct for this effect, dilution of the sample is recommended. This, unfortunately, has the disadvantage of decreasing the sensitivity, so that the low detection limits of the ICP-MS are partially sacrificed [3]. However, with the Perkin Elmer Sciex Elan 5000A ICP-MS instrument accurate and precise analyses can be made with a total dissolved solids content of 10% or less with few matrix effects, specifically because of the design of the instrument. The majority of our solutions were normally analyzed in their undiluted form.

Correction for matrix effects can also be made by using an internal standard, ie, an element added in a known and equal concentration to all the samples and standard for analysis. The signal for the analyte element(s) in both sample and standard is divided by the signal for the internal standard, and this ratio is then used as the analytical signal. This allows for correction of random changes in the signal and it can also be used to correct for systematic fluctuations of the analytical signal in the sample and standards due to matrix effects. At the same time this improves the precision of the measurements [7]. Accurate correction for matrix effects is possible only if the internal standard (normally Y, In, or Rh) is chosen with a mass number as close as possible to that of the analyte element(s). If the analyte elements are spread over a wide mass range, one internal standard will not be sufficient, rather, several internal standards will have to be selected. For our analyses, four internal standards, Sc, Y, Rh and Ta, were used to cover the mass range of elements. For our quantitative analyses, Sc was used to correct for elements in the mass range 11 to 65 (ie, B, Al, Ti, V, Cr, Mn, Co, Zn), Y for the mass range 77 to 89 (ie, As, Se, Sr), Rh for the mass range 103 to 137 (ie, Ba), and Ta for the mass range 181 to 209 (ie, Bi). The analysis of TPN solutions presented a unique matrix never before analyzed with ICP-MS to the knowledge of the author. This offered unique challenges to the analyst and researcher alike.

Instrument operation

For analyses, the Perkin Elmer Sciex Elan 5000A ICP-MS parameters were as follows: plasma flow of 15 L/min, nebulizer flow 0.9 L/min, RF power 1000 Watts, CEM voltage 3.8 kV, and sample uptake 1 mL/min. For quantitative analyses, the instrument was set for 20 sweeps/reading, with 1 reading and 5 replicates. Dwell times were set for 35ms. Dwell times of 175ms were set for Ti, Se, and B. These were elements which had relatively low values in the TPN solution in relation to the others. For "Total Quant" analyses, the instrument was set for 15 sweeps/reading, with 1 reading, 1 replicate, and a dwell time of 20ms per mass analyzed. Table 2-1 contains selected ions and correction equations used for quantitative analyses of complete TPN solutions.

The ICP-MS instrument is kept in an ultra clean trace element-free room utilizing a system of positive pressure and a high efficiency particulate air (HEPA)-filter to prevent dust contamination and allow for the detection of very low concentrations of trace elements. Other environmental controls include an air cooling device in the room housing the ICP-MS to maintain a constant temperature of the instrument while operational, thereby preventing instrumental drift. The temperature of the room is maintained within 21 ± 1°C.

Quality controls

Preparation of standards and samples for analysis

Prior to analyzing any samples, various standards and quality control samples were prepared. This was performed in ultra-clean rooms utilizing a system of positive pressure and (HEPA)-filtered air. A mixed working standard containing all the elements of interest for quantitative analysis (from which instrument calibration standards were made), a mixed internal quality control standard (in which different concentrations were used to maintain the calibration curves of the elements chosen for quantization), and a mixed internal standard (with the four elements to cover the mass range being analyzed, ie, Sc, Y, Ta, Rh) were made from individual ultra pure standard ICP-MS solutions from SPEX (Park Avenue, Edison, NJ). SPEX standard solutions come with a certificate of purity, are National Institute of Standards and Technology (NIST) traceable and are ISO (International Standards Organization) 9001 approved. An external quality control NIST Standard Reference Material (SRM) aqueous sample (1643C water) was prepared as a calibration check for the instrument. NIST SRM's come with a certificate of quality and two year guarantee. Thus, the instrument calibration was maintained with the gold standard of international quality control. The SPEX and NIST SRM used for this project represented extremely high calibre quality controls.

All blanks, standards, and samples were prepared for analysis in 0.5% nitric acid. Seastar certified trace metal-free (TMF) nitric acid, double sub-boiled distilled in quartz (Seastar, Co. Vancouver, BC) was used for all analyses. Trace element-free Nanopure water (Barnstedt Nanopure D4700 Deionizer System equipped with one macropure [D0836], one organic-free [D5021], and two ultrapure DI [D0809] cartridges and a 0.45 μ cross-flow filter [D3751] to produce 18.0 megaohm trace element-free deionized water) was used for all dilutions.

After instrument calibration, at the beginning of the analysis, two TMF water washes were run through the instrument. The internal quality control sample standards were run as a calibration check, followed by the NIST external quality control samples. The TMF aqueous blank was then run (this value was subtracted from all of the other values), followed by ten samples. After every ten samples, the internal quality control samples were run to maintain the calibration curve of the elements. Midway during the analysis of samples, a calibration was performed, followed by another TMF aqueous blank reading. A computerized automated system was used to input the samples. Samples were aspirated for 45 seconds with help of a peristaltic pump into the sample uptake system, which allowed enough time for samples to reach the nebulizing system. Between samples, the uptake tubing was washed for two minutes with Nanopure water.

Contamination Control For Trace Element Analysis

During this project, scrupulous efforts were undertaken to ensure samples were protected from both the introduction of trace element contaminants as well as the loss of intrinsic elements of interest. Only the purest chemicals and water were accepted for the project. Water for dilution, as well as that used for cleaning procedures, was trace element-free Nanopure water (Barnstedt Nanopure D4700 Deionizer System as described previously). Sample containers and equipment were washed with an approved cleaning solution for trace element analysis (Extran 300; BDH Chemicals, B80002), and were treated with a good ultrapure leeching agent (HNO_3; Baker Company, Instra-analyzed for trace metal analysis, 9598-00) to leech impurities from the surfaces.

Storage containers may differ greatly with respect to adsorption of trace elements leading to loss of analyte, or contamination by the leeching of trace elements from the tube walls [8]. High density polypropylene sample tubes (Sarstedt, St. Laurent, Quebec) which have been used with much success by the Environmental Toxicology/Trace Elements Laboratory, were utilized for all samples in this study. To further minimize influences on trace element concentration by adsorption or leeching, sample freezing has been recommended [8]. Samples which were not able to be immediately analyzed during this study were frozen at -70°C.

Prior to beginning trace element experimentation for this project, a study was conducted to ensure the integrity of the laboratory environment for trace element analysis. The results of the experiment allowed us to establish that our laboratory facilities and choice of sample tubes were acceptable for trace element analysis, and that our two main choices of water sources were free of trace element contamination. The extensive training and guidance with regard to the acquisition of appropriate supplies and experimental technique by the Trace Elements/Environmental Toxicology Laboratory UAH were crucial to ensuring the integrity of the analytical values obtained in this project.

External quality controls

A rigorous external quality control program is in place at the UAH Trace Elements/Environmental Toxicology Laboratory. The laboratory participates in the (North American) Analytical Products Group (APG) Performance Evaluation Interlaboratory Testing Program and the Alberta Water Analysts Committee (AWAC) Interlaboratory Round Robins. Participants in these programs periodically receive blind aqueous samples which are quantified for trace elements using various analytical instruments. Each laboratory sends its results to the Analytical Products Group (Belpre, OH) or AWAC, accordingly, which compiles the data and returns a detailed report including a z-score ranking to the laboratories. In addition, the external international NIST SRM 1643C water was run on each analytical day during this project to verify calibration, as described previously. A constant check on instrumental quality control is, therefore, maintained. Table 2-2 contains NIST SRM 1643C water certified values and measurements obtained with the ICP-MS.

The results of APG and AWAC external quality control reports reviewed during the period of sample analysis for this project, demonstrated the excellent accuracy and detection capabilities of the UAH ICP-MS analyses at both high and low concentrations of trace elements.

For the elements of interest in this project, all of the z-scores from the external quality control programs were well within acceptable ranges for a wide variety of trace element concentrations. (Unfortunately, no reports of B, Ti, Sr and Ba were available.) Table 2-2 shows the certified values and ranges of the SRM 1643C water and the average values measured by the ICP-MS during quantitative analyses performed in this study. The average measurement considering all of the analytical days indicates that most elements were within the certified reference range and very close to the certified values. Two days are also shown in which the SRM 1643C water was analyzed twice along with the study samples. There was little difference in measurements within days as well as between different analytical days. We are thus, confident in the sensitivity, accuracy, and precision of the analytical method being used in this study for the analysis of trace elements.

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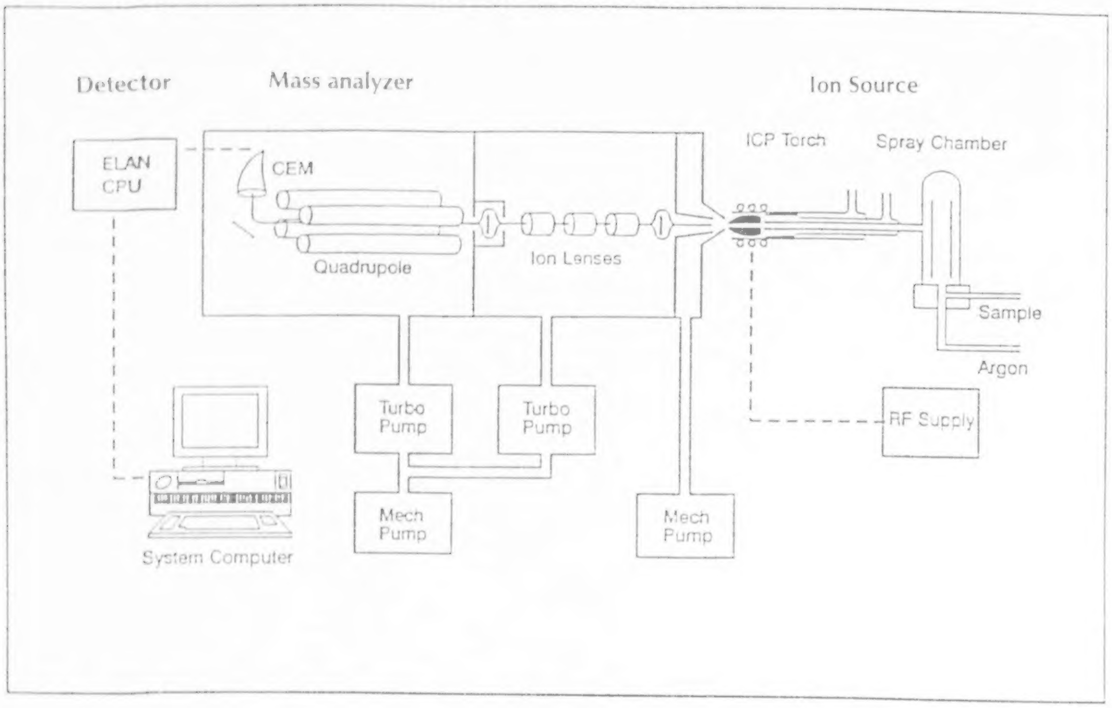
Detector
Mass analyzer
Ion Source
ICP Torch
Spray Chamber
ELAN CPU
CEM
Quadrupole
Ion Lenses
Sample
Argon
RF Supply
Turbo Pump
Turbo Pump
Mech Pump
Mech Pump
System Computer

Figure 2-1. Components of the Perkin Elmer Sciex Elan 5000A ICP-MS instrument. Courtesy of Perkin Elmer, Toronto, ON.

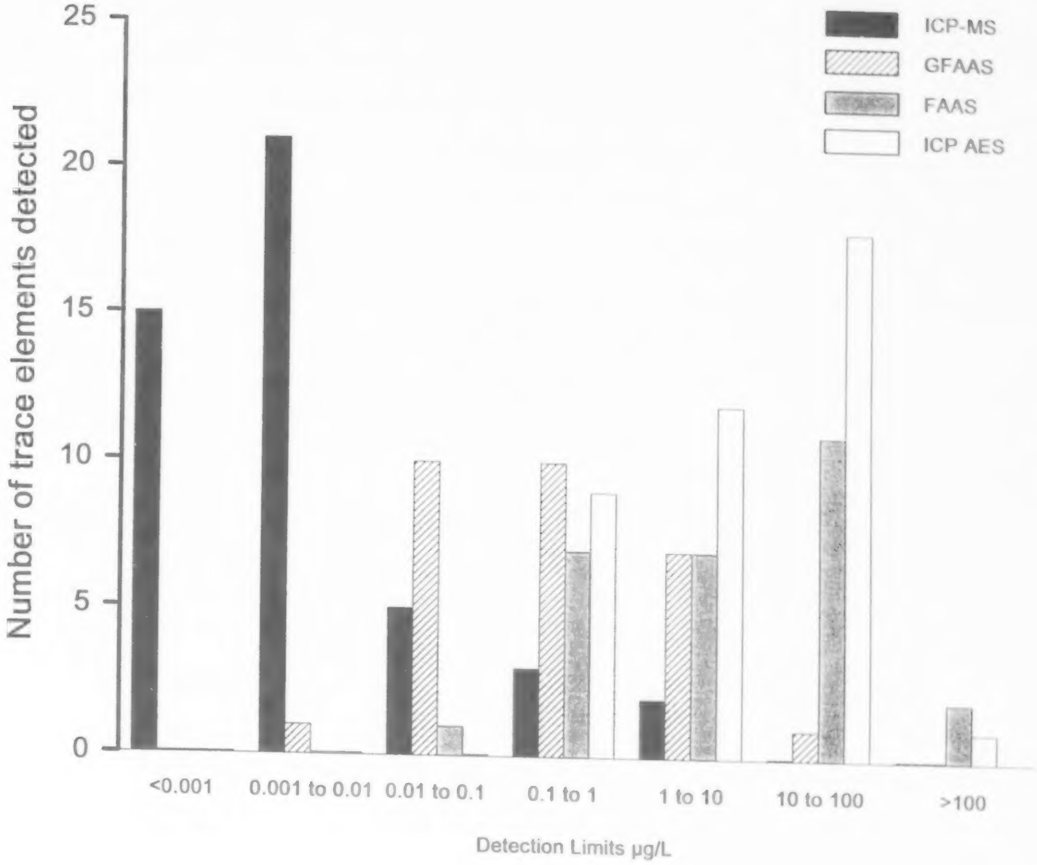

Figure 2-2. Comparison of detection limits in ICP-MS, GFAAS, FAAS, and ICP AES. Adapted from Vandecasteele, C and Block, CB. Modern Methods for Trace Element Determination. Chichester: John Wiley & Sons, 1993: 171.

Table 2-1. Selected ions and correction equations employed for quantitative ICP-MS analysis of TPN solution.

Element	Selected Ion Measured	Correction Equation
B	B^{11}	
Al	Al^{27}	
Sc*	Sc^{45}	
Ti	Ti^{47}	
V	V^{51}	- 0.00204 * Cl^{35}
Cr#	Cr^{53}	- 0.00204 * Cl^{37} - 0.005 * C^{13}
Mn	Mn^{55}	
Co	Co^{59}	- 0.00204 * Ca^{43}
Zn	Zn^{64}	- 0.04117 * Ni^{60}
Cu	Cu^{65}	
As	As^{75}	- 3.087 * Se^{77} +0.9948 * Se^{78}
Se	Se^{77}	
Sr	Sr^{88}	
Y*	Y^{89}	
Rh*	Rh^{103}	
Ba	Ba^{137}	
Ta*	Ta^{181}	

*Represent internal standards.

#For inorganic component solutions Cr^{52} was measured with a correction for Ar^{40}C^{12}: Cr^{52} - 0.05 * C^{13}.

Table 2-2 Comparison of National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1643C Water with measured values during the study duration

TRACE ELEMENT	SRM CERTIFIED VALUE (μg/L)	SRM REFERENCE RANGE (μg/L)	ANALYSIS DATES / VALUES OBTAINED[1]				AVERAGE MEASUREMENT ALL DAYS[3] (μg/L) ± s.d.	n = DAYS
			Mar 29/95 (μg/L) (s.d.)[2]	Mar 29/95 (μg/L) (s.d.)	Apr 5/95 (μg/L (s.d.)	Apr 5/95 (μg/L (s.d.)		
Zinc	73.9	73.0 - 74.9	64.8 (1.5)	67.8 (1.2)	70.1 (2.4)	70.2 (3.5)	65.6 ± 18.9	12
Copper	22.3	19.5 - 25.1	20.5 (0.4)	21.1 (0.5)	22.1 (0.4)	21.7 (3.6)	21.2 ± 2.6	12
Manganese	35.1	32.9 - 37.3	34.7 (0.7)	34.1 (0.7)	36.4 (1.0)	36.9 (0.8)	36.7 ± 2.7	12
Chromium	19.0	18.4 - 19.6	18.3 (0.3)	18.6 (0.3)	19.6 (0.8)	19.4 (0.6)	19.5 ± 1.5	12
Selenium	12.7	12.0 - 13.4	12.9 (0.6)	11.7 (0.9)	13.3 (0.8)	8.0 (1.4)	11.9 ± 1.8	12
Boron	119.0	117.6 - 120.4	102.8 (2.7)	124.7 (2.3)	116.5 (3.2)	113.9 (2.5)	118.3 ± 8.5	12
Aluminum	114.6	109.5 - 119.7	123.4 (2.5)	126.8 (2.9)	121.6 (3.2)	118.6 (2.2)	122.1 ± 7.9	12
Titanium			7.8 (0.2)	8.0 (0.1)	8.2 (0.1)	7.3 (0.7)	8.4 ± 0.7	12
Vanadium	31.4	28.6 - 34.2	30.0 (0.5)	29.9 (0.3)	31.8 (0.6)	30.6 (1.5)	31.8 ± 2.0	10
Cobalt	23.5	22.7 - 24.3	23.9 (0.3)	23.0 (0.5)	24.8 (0.7)	25.2 (0.5)	25.2 ± 1.8	10
Arsenic	82.1	80.9 - 83.3	84.5 (3.2)	86.0 (2.7)	86.1 (1.9)	77.2 (1.9)	80.8 ± 6.4	10
Strontium	263.6	261.0 - 266.2	259.4 (2.7)	263.7 (2.7)	266.6 (5.5)	262.7 (2.8)	263.5 ± 4.8	10
Barium	49.6	46.5 - 52.7	52.0 (0.8)	48.3 (1.0)	53.5 (0.8)	51.5 (1.0)	51.2 ± 4.2	12
Bismuth			23.5 (2.0)	15.1 (1.1)	20.6 (1.4)	————	19.6 ± 6.2	8

[1] Two days chosen for direct comparison of trace element measurement within days and between days using ICP-MS.

[2] Standard deviation (s.d.) of the ICP-MS analysis.

[3] Average of all days in the study in which elements were measured quantitatively.

CHAPTER 3

INDIVIDUAL COMPONENTS OF THE TPN SOLUTION ARE CONTAMINATED WITH TRACE ELEMENTS

SPECIFIC AIM

The specific aim of this experimental section was to determine the baseline level of trace elements in each of the components comprising a typical TPN solution.

INTRODUCTION

It is now widely recognized that trace elements are vital to the management of patients receiving TPN. TPN component solutions are assumed to be free of trace elements, the exception being those specifically manufactured as trace element additives. However, a number of the components used for TPN solutions have been shown to be contaminated with trace elements such as Zn [1,2,3,4,5,6], Cu [2,3,5,6], Mn [1,3,7], Cr [8], Se [5,9], Al [10,11], and more recently B and V [12]. Recommendations for addition of the essential trace elements Zn, Cu, Cr and Mn to TPN solutions have been made by the American Medical Association Expert Panel [13]. Ironically, these same trace elements are of the frequently reported contaminants in several intravenous commercial components as listed above. The concentrations of trace element contaminants have been found to vary significantly between various types of components, between manufacturers and also between component lots from the same manufacturer [2,4,14]. There have been few reports regarding intra-lot variation of trace element contaminants since for most elements it has been demonstrated to be minimal when compared to inter-lot variation.

The potential sources of trace element contamination of TPN components are numerous and include packaging, manufacturing methods, and inherent impurities in the chemical constituents used to produce the components. Several intravenous components are packaged in plastic containers. Plastics contain a number of chemical additives which consist of trace elements. For example, Zn compounds are commonly added to plastics to assist in molding and prevent sticking to the manufacturing equipment, and Sn salts are often used as stabilizers [15]. Since plastic packaging is not totally inert, trace elements can be extracted into the contents of the container. Trace element contaminants occasionally emerge in all types of plastics as a result of anomalies in the production process affecting certain batches. Most plastic storage bags, like those used to hold sterile water, dextrose, and amino acid solutions, are constructed from polyvinyl chloride. Polyvinyl chloride has been shown to be contaminated with approximately nine elements, the major ones being: Fe, Zn, and Sb [16]. Metal equipment is used in the manufacture of plastic materials, such as polyvinyl chloride bags. It is possible that trace elements contaminants in the metal alloys may be leeched into the plastic in contact with the metal during manufacturing. These trace element contaminants may, in turn, leech from the bags into the components upon contact and end up in the compounded TPN solutions prepared for patients.

To provide closures for intravenous fluid bottles and multiple dose sites, rubber is the material of choice. A large variety of fillers and other chemical aids are used in the production of rubber. Zinc-oxide compounds are used as vulcanizing agents, activators, and reinforcing fillers; Ba is used as an extending filler; Pb, Ni and Cr salts are used as inhibitors; and Ti is employed as a pigment agent [15]. The rubber stopper has been implicated as a source of Zn, Cr, Fe, Ni and other heavy metals [1,17]. A twelvefold difference in the amount of Zn leeched from rubber stoppers (10.75 to 132.00 μg) was reported by Jetton et al [1]. vanCaillie et al [5] examined rubber stoppers from component stock bottles for Zn, Cu, and Se contamination. All rubber stoppers were found to contain a significant amount of Zn (9.2 to 29.4 mg/g rubber). No contamination of the stoppers with Cu or Se was reported. The extent of trace element contamination from rubber closures likely depends upon variables such as duration of exposure of the component to the stopper and shaking during shipping and storage [5]. Unfortunately, each manufacturer has their own formula and, thus, the seals or stoppers may differ greatly in composition. There is also a danger that individual lots of rubber stoppers from the same manufacturer may vary in composition according to the availability of ingredients. Specialist suppliers have helped reduce this source of variability considerably but have not succeeded in eliminating it [15].

In general, components stored in glass containers are more vulnerable to trace element contamination than are those stored in plastic. Glass is a common packaging material for a number of the TPN components. However, the materials used to manufacture glass for parenteral components are often of questionable purity. The sand, alumina and limestone used to make glass likely contain elements other than Si, Ca and Al [15]. The glass manufacturer may add B, Ba, or Zn salts; Ti, Fe and Mn salts are added to make colored or opaque glasses.

Borosilicate glass, like that used to store a number of TPN components, has been shown to be contaminated with about 14 elements, the major ones being Si and B [16]. Additionally, glassware may contain variable inter-lot amounts of leechable trace element impurities depending on the purity of the materials obtained for the manufacture of particular batches. Trace elements may be leeched from the glass containers during storage of the parenteral product.

During manufacturing, component solutions are filtered prior to packaging in their respective containers. The membrane filters used for this purpose are made from cellulose esters, polycarbonate, synthetic polymers, silver or stainless steel [18]. Polycarbonate has been shown to contain approximately 10 elements, the major ones being Cl, Br and Al [16]; stainless steels are alloys of Fe and C with mainly Cr and Ni and traces of other elements such as Mn [19]. Trace elements contained in the filter materials may leech from the filters into the final component solutions. After filtering, heat stable parenteral components are frequently terminally sterilized in the final glass and plastic containers. The heat sterilization process accelerates the chemical attack on the glass and plastic which may release trace element contaminants into the component solution [18]. Water is used as the solvent in most TPN components. The pharmaceutical manufacture of water employs glass and stainless steel equipment. It is possible that the trace elements present as impurities in glass and steel, as discussed, are leeched into the sterile water used as solvent, which can then be transferred to TPN components.

A primary source of trace element contaminants are the chemical constituents used to manufacture the various electrolyte, carbohydrate, and protein solutions. Manufacturers obtain chemical constituents from a number of sources. Depending upon the geographical location and the types of contaminants co-existing with the chemicals at various mining sites, the trace element contamination can be extremely variable. This may partially explain the differences in trace element contamination observed between manufacturers of TPN components. Unless specific trace elements are consistently tested for in different batches of chemicals purchased from suppliers as part of quality control measures, there is no way to be certain that each product produced is free of all trace element contaminants.

Long-term TPN patients can inadvertently accumulate significant amounts of trace elements due to contamination of component solutions. Since over-infusion of trace elements in TPN solutions may lead to toxicity, a knowledge of the trace element concentration of various commonly used component solutions is crucial.

In the past, the analysis of individual TPN components for every possible trace element contaminant would have been considered a nearly impossible, if not infinitely time consuming. However, new technology has enabled us to scan the entire periodic table of the elements and, thus, determine which trace elements and what quantities may be present in various TPN components. This study is unique in that it is the first to simultaneously examine TPN components for numerous trace element (ie, 66) contaminants and quantify them using the multi-elemental technology of ICP-MS.

The purpose of this study was to determine the extent to which trace elements are present in expected amounts (ie, confirm labelled levels) and as contaminants of components used to prepare TPN solutions. Additionally, it examined the degree of inter-lot variation exisiting in each component from its respective manufacturer. Nine different components typically used in the formulation of TPN solutions were examined. Solutions were scanned for 66 trace elements to determine the presence and quantity of trace element contaminants.

MATERIALS AND METHODS

Supplies

Nine components used in the formulation of standard TPN solutions were selected for determination of trace element concentration. Three different lots of each component from the same manufacturer were purchased from University of Alberta Hospitals Pharmacy over a period of one year as the inventory turned over. (Table 3-1). Adequate volumes of Lot 1 components were purchased for use in the delivery study described in Chaper 5. Due to a change in pharmacy suppliers during the study, a different manufacturer was represented in one of the purchased lots of Ca-gluconate and NaCl solutions. As a result of the limited turnover of the MTE-6 trace element concentrate, only two lots were examined during the study.

Lot 1 of NaCl and Ca-gluconate were supplied by International Medication Systems (IMS) Limited, Mississauga, Ontario. Lots 2 and 3 of NaCl and Ca-gluconate, and both lots of MTE-6 were supplied by Lyphomed, Markham, Ontario. Potassium chloride (KCl) was supplied by Abbott Laboratories Limited, Montreal, Quebec. Travasol, dextrose, and sterile water were supplied by Baxter Corporation, Toronto, Ontario. Adult MVI and $MgSO_4$ were supplied by Sabex, Boucherville, Quebec.

Sample Collection

Using non-powdered gloves as a precaution against extraneous trace element contamination, samples were prepared for analysis in a laminar air flow hood equipped with HEPA-filter. Samples from each stock component were transferred to KIMAX Class A glass volumetric flasks (Kimble Glass, 28013) from their commercial containers using plastic disposable pipette tips (Clear Metal Free Flex-Tip 1-100μL and Blue Tips 101-1000μL; Brinkmann Instruments Inc., Westbury, N.Y. 11590) with Eppendorf Digital Pipettes (Brinkmann Instruments Inc. Westbury, N.Y. 11590) or class A volumetric pipettes (Fisher Scientific, Ottawa, Ontario) as required. Remaining portions of components were transferred to clean, new high density polypropylene containers and stored as recommended by the manufacturers for remaining analyses. Volumetric flasks were previously soaked in Extran 300 concentrated cleaning solution (BDH Chemicals, B80002) over-night and rinsed well with Nanopure trace element-free water (as described in Chapter 2). Twenty-five millilitres of pure nitric acid (Baker Company, Instra-analyzed for trace metal analysis, 9598-00) was added to each flask and swirled. The flask was rinsed well with Nanopure trace element-free water. Flasks were left to dry in the HEPA-filtered laminar air flow hood. Glass volumetric pipettes were prepared for trace element analysis similarly to the volumetric flasks as described above.

The quantity of sample taken from each of the nine components was calculated according to its relative proportion used to compose a one litre standard TPN solution (Table 3-2). This was done to account for the differences in volume of the components used to formulate TPN solutions. Samples were diluted with Nanopure trace element-free water according to this ratio. Diluted samples were mixed well, transferred to 30 mL polypropylene tubes (Sarstedt, St. Laurent, Quebec) and were analyzed immediately for trace element content. Samples were randomly obtained from one bag/bottle/vial of component solution per lot due to the lack of reported intra-lot variation in trace element contamination.

Trace Element Analysis

Samples were analyzed for trace elements via inductively coupled plasma-mass spectrometry (ICP-MS) (Perkin Elmer Sciex Elan 5000A ICP-MS System, Perkin Elmer, Toronto, ON). Analyses were performed in two parts. First, a "Spectrum-directed" analysis of 66 trace elements was performed employing the Total Quant Mode (scanning from atomic mass 3 [Li] to 92 [U]; major elements were excluded) to screen for existing trace element contaminants (Table 3-3). A Spectrum-directed analysis was performed on one sample from each component in each of the three lots. Any trace elements appearing at a level $\geq 1 \mu g/L$ were considered to be present at a level sufficient to warrant specific "Element-directed" quantitative analysis employing the Quantitative Mode of the ICP-MS. Element-directed analysis was performed approximately two weeks after the Spectrum-directed screen on three or four samples taken from each of the three lots of the nine components. Separate lots were analyzed over a period of one year as lots turned over in the Pharmacy inventory. Samples within each lot were analyzed within one week. Chromium concentrations were difficult to quantify (with the ICP-MS instrument available to us) in the Travasol and dextrose components due to the interfering organic products present. Therefore, Lot 1 samples of Travasol and 70% dextrose were analyzed by graphite furnace atomic absorption spectrometry (Quebec Centre of Toxicology) and an instrumental correction factor was applied to these two solutions obtained as Lots 2 and 3 analyzed by ICP-MS. Even though I (Iodine) was part of the MTE-6 additive, it was not analyzed during this experiment because the ICP-MS instrument available for analysis was not yet configured to measure anionic species.

Statistical Analysis

Results are reported as means ± standard deviation of trace elements per litre of TPN solution (ie, $\mu g/L$). An analysis of variance, followed by the Student-Newman-Keuls test where appropriate, were employed to identify significant differences using the SigmaStat statistical software package (Jandel Scientific, San Rafel, CA). T-tests were used to identify significant differences in measured trace elements in MTE-6 from expected (labelled) values. A probability value of $p < 0.05$ was considered to be significant.

RESULTS

Trace element contamination in components expected to be free of trace elements

Spectrum-directed analysis revealed the presence of twelve trace element contaminants, Zn, Cu, Mn, Cr, Se, B, Al, Ti, Ba, V, As, and Sr, in the TPN components analyzed. Trace element contaminants were present in all components, with different trace elements contaminating at various concentrations (Figures 3-1 to 3-8). Boron and Al were present as contaminants in every component analyzed. Travasol, KCl, Ca-gluconate, and NaCl contained the greatest number of trace element contaminants, while the least number were present in sterile water and $MgSO_4$. The percent contribution of trace elements in each component in the three lots, to the total concentration of trace elements in a TPN solution (based on Table 3-2) is presented in Tables 3-4a to 3-4c.

Trace element contamination in MTE-6

In both Lots of MTE-6, the measured concentration of trace elements was significantly greater than the expected concentration (labelled amounts) from the manufacturer. The exceptions were Cr and Se concentrations in Lot 2. Chromium was not significantly different from the expected concentration and Se was significantly lower than the expected concentration ($p<0.05$) (Figure 3-4).

Inter-lot variation of trace element contamination

Significant inter-lot variations in trace element contamination were present in components from the same manufacturer (Figures 3-1 to 3-8). In NaCl and Ca-gluconate components, in which more than one manufacturer was represented, there was significant variation in the trace element contamination between manufacturers of similar solutions. In NaCl, Sr contamination in Lot 3 (Lyphomed) was significantly less than in Lots 1 and 2 (IMS) ($p<0.04$) (Figure 3-1). In Ca-gluconate, no Mn contamination was present in Lot 3 (Lyphomed), which made it significantly different from Lots 1 and 2 (IMS) which contained Mn ($p<0.008$) (Figure 3-5).

To provide clinical relevance to the amounts of trace element contamination, we compared the expected concentration of trace elements that would be present in two litres of TPN solution to the concentrations in a TPN solution calculated from the values obtained for Lots 1 and 2 components (based on Table 3-2). Since there were only two lots of MTE-6 analyzed, only TPN solutions based on Lot 1 and 2 components were represented. A volume of two litres of TPN solution was chosen to reflect an average patients' nutritional needs as well as meet AMA guidelines for the parenteral addition of trace elements. In general, calculated TPN solutions contained a greater concentration of trace elements than the expected amounts present in a standard TPN solution and absorbed from the daily oral diet (Table 3-5).

DISCUSSION

This study demonstrates that trace elements are present in significant quantities in TPN components. The concentrations of trace element contaminants in the TPN components analyzed varied between different lots from the same manufacturer, and between components from different manufacturers, as previously described in the literature. Calculations with the use of a standard TPN solution (Table 3-2), demonstrate that the inadvertent intake of ultratrace elements from TPN solutions may be substantial but inconsistent because of the variable degree of contamination (Table 3-5).

Zinc

Zinc is a notorious contaminant of many TPN components (Table 3-6). Zinc was present as a contaminant in six of the nine components analyzed in this study with the highest concentrations existing in Travasol (Figure 3-3) and MVI (Figure 3-6). Variation in Zn contamination has been attributed to Zn leeched from rubber stoppers [1]. Zinc is a frequent contaminant of rubber, glass, as well as of water and many chemicals, even those of the highest analytical grade [20]. It is, therefore, not surprising that the Zn contamination of TPN components is so variable. The Zn concentrations of TPN solutions calculated from Lots 1 and 2 components were 17 to 20% greater than the expected concentration because of contamination (Table 3-5). The expected concentration of Zn in the TPN solution was based on the AMA parenteral recommendation of 2500 to 4000 μg/day Zn in stable adults [13]. It is unlikely that this level of Zn contamination would produce toxic effects in patients who have normal renal function. Many patients actually need increased amounts of Zn to replace excess gut losses which occur in various gastrointestinal diseases.

Copper

Copper was not a major contaminant of the TPN components analyzed in this study and was only present in Travasol (Figure 3-3) and sterile water (Lot 1) (Figure 3-8). Copper appears to be present only in small concentrations in most TPN components (Table 3-6). However, amino acids can contribute substantial amounts compared to other components. The precise source of Cu contamination is unknown. It could originate as a result of the chemicals, storage containers or manufacturing processes as outlined earlier. The Cu concentrations in the calculated TPN solutions in Table 3-5 are 7 to 13% greater than the expected amounts based on AMA parenteral recommendations of 500 to 1500 μg/day Cu in a standard TPN solution [13]. There is little concern for Cu toxicity as a result of this level of contamination. However, hepatic accumulation and damage are possible long-term consequences of chronic administration of Cu above the biological requirements of patients [21].

Manganese

Manganese does not appear to be a major contaminant of most TPN components when concentrations are expressed in terms of standard volumes added to TPN solutions, as demonstrated by this and other studies (Table 3-6). Very low levels of Mn contamination were found in four of the nine components in this study. The highest concentration was in $MgSO_4$ (Figure 3-7). Rubber stoppers have been implicated as a source of Mn contamination of TPN components [1]. However, in the study of Kurkus et al [7] Mn values varied from undetectable to 17.0 μg/L in component solutions in bottles with rubber stoppers. It is more likely that contamination occurs as a result of manufacturing processes or the raw materials used [7]. Manganese is unique among the trace elements of established importance in human nutrition in that no Mn deficiency syndrome has been described in patients fed parenterally [22]. Neverthelesss, Mn is one of the essential trace elements which are routinely added as part of TPN regimens, and for which published recommendations have been available from the AMA since 1979. The relative safety of supplemental Mn depends on the effectiveness of the body's homeostatic mechanisms, which are at the level of intestinal absorption and Mn excretion via the biliary tract. In patients receiving TPN, the first of these homeostatic mechanisms is bypassed. If cholestatic liver disease is present, the second mechanism is likely to be impaired or lost [22]. In this study, the Mn concentrations calculated from Lots 1 and 2 components were only 7 to 9% greater than the expected concentration (Table 3-5) in a standard TPN solution based on the AMA recommended daily intravenous intake for stable adults of 150 to 800 μg/day [13]. The Mn concentrations in the solutions represented in Table 3-5 would not pose a risk of toxicity in most circumstances unless a physiological condition, such as cholestasis existed in which excess Mn could not be fully excreted [22].

Chromium

In general, the Cr contamination of the nine TPN components studied was minor. The results of other studies also indicate that Cr contamination of TPN components is low (Table 3-6). Results of graphite furnace atomic absorption spectrometry indicated that the Cr content of Travasol (Figure 3-3) and dextrose (no figure) in Lot 1 was minimal, ie, <2.0 μg/L. Ca-gluconate (Lot 1) (Figure 3-5; Table 3-4a) and KCl (Lot 3) (Figure 3-2), however, displayed substantial contamination. Ito et al [8] analyzed complete TPN formulas prepared for inpatients for Cr contamination. Contamination with Cr ranged from 1.1 to 5.9 μg Cr per formula (above that in the Cr additive). This demonstrates that substantial and variable amounts of Cr can be added by the TPN components. The definite source of the Cr contamination of TPN components is unknown. It is possible that Cr was leeched from the metal alloys used in the manufacture of the sterile water solvent, as well as have been present as a contaminant of the chemicals used to manufacture the solutions. Table 3-5 indicates that Cr contamination of TPN solutions calculated from Lots 1 and 2 components is variable and that the Lot 1 solution was 136% of the expected amount based on the AMA guidelines of 10 to 15 μg/day for stable adults [13]. Toxicity is unlikely to result, even at approximately two times the recommended parenteral level, if adequate homeostatic mechanisms (ie, adequate renal function) are present. However, the significance of increased Cr exposure as a result of contamination of TPN solutions is not known.

Selenium

There is general agreement among most trace element researchers that TPN solutions contain negligible quantities of Se [23]. This is demonstrated in Table 3-6. Small amounts of Se contaminated three of the components in this study: Travasol (Figure 3-3), NaCl (Figure 3-1), and KCl (Figure 3-2). In contrast, Smith and Goos [9] found that certain components contained significant amounts of Se, the major source being dextrose (Table 3-6). The Se content of various dextrose solutions tested did not correlate with the dextrose concentration, implying that the source of the Se was not the sugar, but rather the manufacturing process. The exact source of the Se contamination of TPN components remains ambiguous. Smith and Goos [9] suggested that the high concentrations of Se in dextrose may potentially provide enough Se to become toxic with continued administration. The calculated TPN solutions in Table 3-5 indicate that patients on long term TPN would receive 31 to 43% greater amounts of Se than expected from the standard TPN formula. However, the amount present in the solutions does not appear to be excessive based on guidelines for the addition of 40 to 120μg/ day to home TPN formulas [24].

Boron

There have been few reports which have examined B contamination of TPN components. In this study, B was present in each of the components as a contaminant. The highest concentrations were observed in dextrose and Ca-gluconate (Figure 3-5). The one study which has examined the B content of various TPN components suggests that contamination with B is highly variable (Table 3-6). Boron is present in glass and may be leeched into TPN component solutions with prolonged storage. Boron may also have be present in the chemicals used to prepare TPN components. Since B is not yet a proven essential nutrient and possesses no recommendations for parenteral addition, it is an unexpected contaminant of TPN components. Therefore, the TPN solutions calculated from Lots 1 and 2 components in Table 3-5 contain greater than 100% of the expected concentration of B compared to a standard TPN formula. The quantity of B present in the calculated TPN solutions was within the amount absorbed from the daily oral diet (Table 3-5). The significance of this element in TPN solutions is not known.

Aluminum

Aluminum contaminated all of the TPN components examined in this study. The highest levels were present in Ca-gluconate (Figure 3-5), Travasol (Figure 3-3), and sterile water (Figure 3-8). The Al content of a number of commercially available components used in TPN solutions has been found to vary widely (Table 3-6) but Ca salts have consistently been the most highly contaminated components of TPN solutions [25]. Our results, in addition to the studies summarized in Table 3-6, tend to agree with this observation. The reasons for Al contamination of TPN solutions remains largely unknown. In the case of Ca salts, the use of less than ultrapure reagents may lead to the failure to remove natural contaminants, while the production process may introduce additional Al contaminant to the salt. Since some products, such as Ca gluconate, from a number of manufacturers have similar degrees of Al contamination, the source of the contamination may be from the Al naturally present in the chemicals. Variability of Al in other products from different manufacturers would suggest either a variation in Al contamination of various sources or in the manufacturing processes [26]. There is no known physiological role for Al in humans. However, the toxicity of Al in humans is well-established. Aluminum loading and toxicity have been reported in adults and infants who have received Al-contaminated TPN solutions [27]. Regarding the known toxicity of Al to the brain, bone, and hematopoetic system in patients receiving long term TPN, it is the conclusion of several reports that the degree of Al contamination of such solutions should be reduced [11,26]. In the study by Koo et al [26], calculated TPN solutions made from high Al components were shown to contain up to 300 μg/L Al. The Ca-gluconate was shown to contribute over 80% of the total Al load. If the Al contamination were eliminated from the few high Al TPN components (eg, Ca-gluconate), the final Al content of solutions would be significantly reduced, thereby diminishing its potential toxic effects. Koo et al [26] speculated that such a step could reduce the Al load to approximately 12 μg/L. Since Al was an unexpected contaminant of the TPN components, the concentrations of Al in TPN solutions calculated from Lot 1 and 2 solutions components were greater than 100% of the expected amount in a standard TPN solution (Table 3-5). The normal adult kidney can usually excrete such elevated amounts of Al. However, in cases of impaired renal function, Al toxicity can develop. Reports of high Al levels in TPN solutions have appeared in the literature as early as 1970. However, given the results of this study, it does not appear that much has been done to remedy this situation despite the overwhelming research evidence presented.

Titanium

Titanium has not been previously reported as a contaminant of TPN components. Part of the reason for this has been the lack of extensive testing of components for trace element contaminants. Small amounts of Ti were present as a contaminant of Travasol (Figure 3-3) and MVI (Lots 2 and 3) (Figure 3-6). The source of the Ti contamination is unknown. Titanium is present in metal alloys. It is possible that Ti was leeched from the metal equipment in contact with plastic bags during manufacturing, later to be leeched into the Travasol stored in the bags. Titanium could also have been present in the chemicals used in the manufacturing of the parenteral solutions. Titanium was an unexpected contaminant of TPN solutions calculated from Lots 1 and 2 components, as indicated by the greater than 100% difference from the expected amount in a standard TPN solution (Table 3-5). In addition, the quantitiy of Ti present in the TPN solution calculated from Lot 1 components is within the range of Ti absorbed from the daily oral diet, while the quantitiy of Ti present in the TPN solution calculated from Lot 2 components exceeds this range (Table 3-5). Very little toxicological information exists with respect to this element. Its significance in TPN solutions is not known.

Barium

Until recently, no reports of Ba contamination of TPN solutions existed in the literature. One abstract of a French paper indicated that various (unspecified) parenteral nutrition components showed significant concentrations of Ba [28]. Barium was present in three of the components analyzed in this study, with the concentration in Ca-gluconate being the highest (Figure 3-5). Minute amounts were detected in MVI (Lots 2 and 3) (Figure 3-6) and NaCl (Lot 1) (Figure 3-1). Barium is commonly used in the manufacture of rubber. Barium may have leeched from the rubber stoppers of the aforementioned component solutions, or have been present as a contaminant of the chemicals used in the manufacturing of the components. Barium was present as a contaminant of the TPN solutions calculated from Lots 1 and 2 components. Therefore, the concentration of Ba in these solutions were greater than 100% of the expected concentration in a standard TPN solution (Table 3-5). Additionally, Table 3-5 indicates that the quantity of Ba present in the calculated TPN solutions exceeds the amounts thought to be absorbed from the daily oral diet. The long-term effects of exposure to parenteral Ba are unknown.

Vanadium

There have been few reports of the V content of TPN solutions in the literature. In this study, small amounts of V existed in NaCl (Lots 1 and 3) (Figure 3-1) and KCl (Lot 1) (Figure 3-2). Table 3-6 indicates that V may be present in other components such as amino acids, $MgSO_4$ and sterile water. Vanadium is used in the manufacturing of steel and rubber. Hence, V could have been leeched into the component solutions from the rubber stoppers, been a contaminant of the sterile water solvent exposed to steel equipment during manufacturing, or a contaminant of the chemicals used for the manufacturing of the components. Vanadium was an unexpected contaminant of the TPN solution calculated from Lot 1 components (Table 3-5) which resulted in a greater than 100% difference from the expected value of a standard TPN solution. Table

3-5 also demonstrates that V is a variable contaminant of different lots of components purchased from the same manufacturer, and that the quantity of V in the TPN solution calculated from Lot 1 components exceeds the amount absorbed from the daily oral diet. Intravenous V (as V-pentoxide) is lethal at levels of 0.42 mg/kg body weight [29]. The small quantity present in the TPN solution in this study offered little reason for concern.

Arsenic

A very small quantity of As was present in the KCl component solutions analyzed in this study (Figure 3-2). This is an element which is routinely screened for in parenteral products according to United States Pharmacopeia (USP) guidelines. If As was not inherently present in the chemicals at the point of manufacture, it may have been introduced at some point during the manufacturing or storage of KCl. Arsenic was an unexpected contaminant of the TPN solutions calculated from Lots 1 and 2 components. Therefore, it was present at greater than 100% of the expected value of a typical TPN solution (Table 3-5). Arsenic is known for its toxicity. However, in the minute amounts present in the calculated TPN solutions represented it is inconsequential when compared to the significantly greater amounts reported to be absorbed from the daily oral diet (Table 3-5).

Strontium

Strontium has not been previously reported in TPN components in any significant quantities, to the knowledge of the author. Small amounts of Sr were present in Ca-gluconate (Lots 2 and 3) (Figure 3-5) and NaCl (Figure 3-1). It is not surprising that small levels were obtained from Ca-gluconate since Sr tends to exist with Ca in geological formations. Other potential sources of Sr contamination are unknown. Strontium was an unexpected contaminant of the TPN solutions calculated from Lots 1 and 2 components. Therefore, there was a greater than 100% difference from the expected Sr content of a typical TPN solution (Table 3-5). The significance of Sr in parenteral solutions is unknown. However, the small quantities present give little reason for concern considering that 89 to 342 μg are absorbed from the daily oral diet (Table 3-5).

No figure or statistics could be produced for the dextrose component solution because it had to be diluted to a point low enough to minimize the interference of organic compounds with ICP-MS analysis. Therefore, the results should be interpreted cautiously. Results indicated contamination with a number of trace elements: Zn (all three lots; range 13 to 20 μg/L), B (all three lots; range 203 to 560 μg/L), and Al (all three lots; range < 10 to 100 μg/L). The values for Cu, Mn, Cr, Se, B, V, Ti, As, and Sr were reported to be < 10 μg/L. Results of other studies agree with our findings in that there are generally low levels of trace element contaminants in dextrose save for Zn, Al, and B (Table 3-6).

In the past, studies of trace element contamination of TPN components were reported in terms of the concentration contained per volume of the particular component solution. This provides a misleading view of the extent of trace element contamination since very small amounts of highly contaminated electrolyte component solutions are often used in the final TPN formula, thereby, contributing relatively minute amounts to the total. In this study, as in more recent studies, the concentrations of trace elements have been expressed based upon the volumes used in standard TPN solutions; this reflects the important differences in the ratios of component solutions typically used. From Tables 3-4a to 3-4c and Table 3-6, it can be seen that substantial amounts of trace element contaminants are contributed by amino acid and dextrose components. This is likely due to the high volumes of these components added to the final TPN solutions. In contrast, components such as NaCl, KCl, and Ca-gluconate also provide a significant share of contaminants to the TPN solution. This indicates a marked contamination of these components with trace elements. Ca-gluconate appears to contribute significant amounts of Cr, Al, and Ba despite its small percentage of added volume (Table 3-4a to 3-4c). However, the additive effect of the trace element contaminants present in the components to the overall trace element concentration of a TPN solution is one worth noting.

The information presented in Table 3-5 suggests that the concentrations of trace elements in TPN solutions comprised of contaminated components exceeds the expected concentrations of trace elements added according to parenteral guidelines. This indicates that patients may be provided with more trace elements than intended. Additionally, as a result of unexpected contamination of TPN solutions, patients are provided with various "nonessential" trace elements as contaminants. Since results of this study demonstrate that trace element contamination is inconsistent in the TPN components, variations in trace element concentration and contamination will exist in TPN solutions prescribed for patients based on the inter-lot and manufacturer differences.

The guidelines for the addition of trace elements to TPN solutions published by the

AMA Department of Foods and Nutrition in 1979 were based upon the assumption that the TPN solutions were originally free of trace elements. Our results, as well as those of numerous others, clearly contradict this presumption. The clinical implications of trace element contamination of TPN solutions is that patients are exposed to a therapeutic variable (trace element contaminants) that may differ from one bag of prepared TPN solution to another. It only appears to be logical that if TPN solutions are to be used as a source of essential nutrients, ie, trace elements, that the concentration of the elements in the starting components be known. If the starting components contain large quantities of trace elements as contaminants, then subsequent supplementation with trace element additives may prove harmful to the patient. The labelled amounts of trace elements in the MTE-6 component solution of this study appear to be within satisfactory levels. Therefore, the concern for the excess delivery of trace elements is not directed at this component, which appears to be adequately controlled for content, but rather at the other TPN components for which there are few trace element controls.

Patients may actually receive a greater concentration of trace element contaminants if additional TPN components, other than those mentioned in this study, are included in their TPN regimen. For example, intravenous lipids have been shown to be contaminated with a number of trace elements such as Zn, Cu, Cr, Al, B and Mo [4,7,8,12,26,30]. Intravenous lipids were not examined in this study since they were not present in the total TPN solution. Unlike those home TPN patients who receive three-in-one solutions containing lipid, the home TPN patients affiliated with our institution are infused with lipids separately. A later experiment (ie, Chapter 5) using components from Lot 1 was designed to examine trace element alterations occurring in a typical TPN solution in which lipids were not present. It would be interesting, however, to examine the lipid component in a broad study of trace element contamination via a multi-elemental method such as ICP-MS as we have done in this experiment.

This study identified two new trace element contaminants not previously reported to be present in TPN components, namely Ti and Sr. However, the relevance of these and other trace element contaminants in TPN components is difficult to evaluate because of the lack of knowledge surrounding the biological role of trace elements and their potential toxicity to humans. Aluminum contamination of TPN solutions provides an important historical example of the inadvertent provision of trace element contaminants with serious consequences.

What has not yet been clearly addressed, is whether or not the concentrations of the trace element contaminants found in TPN components are altered at some point during the compounding, storage, or delivery of the final TPN solution to patients. It has been suggested that trace element concentrations may be reduced by adsorption or precipitation from TPN solutions. It is also not known whether or not compounding or delivery add additional trace element contamination, which would further exacerbate the existing contamination problem. These questions shall be addressed in the studies that follow.

The notion of contamination is generally associated with deleterious effects for recipients. However, daily oral diets contain a wide variety of trace elements present as natural components of foodstuffs, as well as a result of exogenous contamination during processing and preparation for consumption [31]. It is thought that several trace elements, not yet proven essential, are required in small amounts for various life processes. The presence of trace element contaminants in TPN solutions, like the normal oral diet, may serve as a means of supplying these on a daily basis. It is possible that the contamination of TPN solutions with trace elements, such as B and V, may play a role in the prevention of deficiencies of these elements.

In a number of cases, the results of the trace element contamination of TPN components in this study, are lower than those first reported in the 1970's. It is likely that the differences in concentration are due to the sample contamination which existed in studies of the past. Trace element analyses are extremely difficult due to the necessary controls required to prevent extraneous contamination of samples. Experience and technology have provided numerous guidelines for the control of trace element contamination. Hence, much stricter regulations regarding sampling, cleaning techniques, and equipment choices are adhered to for present-day analytical procedures. Unfortunately, few of the past studies extensively reported contamination controls. Any study which fails to sufficiently report precautions taken against trace element contamination should be interpreted with caution. In this study, appropriate laboratory safeguards against contamination were undertaken. Equipment, water sources, storage containers, and work areas were tested prior to beginning experimentation to ensure freedom from extraneous contamination, as described in Chapter 2.

The United States Pharmacopeia (USP) was created to provide authoritative standards and specifications for pharmaceutical substances and their preparation. Their primary purpose is to assure users of the identity, labelled strength, quality, and purity of pharmaceutical preparations and dictate procedures for the examination of these standards. However, the USP concedes that it is manifestly impossible to include a test for every impurity, contaminant, or adulterant that might be present, and it leaves the responsibility of administering tests suitable for detecting such occurrences to the manufacturers of pharmaceutical products [32]. Unfortunately, most quality control sampling schemes employed by manufacturers are not designed to detect low level trace element contamination because of the small sample size tested [33]. Limit testing guidelines have been established in the USP for the heavy metal (ie, Pb, Hg, Cd), As, and Se content of TPN preparations. Other specific trace element analyses are not required nor performed. Therefore, elements like Ti, Ba, V, Al and Sr are not required to be routinely screened for in pharmaceutical products. The results of this and other studies indicate that there may be a need to add new trace elements such as those outlined above, to the limit testing guidelines. The use of newer multielemental analytical methods like ICP-MS will likely facilitate the screening of manufactured solutions for the presence of trace element contaminants.

CONCLUSION

An enormous amount of information regarding the trace element contamination of TPN solution components has been published over the past two decades. It is not only the quantities and inconsistencies in the amounts of trace elements found in the components that is alarming but also the fact that this contamination has remained for 20 years without any serious action being taken to remedy the situation. Present commercial TPN component solutions cannot be relied upon to provide consistent levels of trace elements. The various components that comprise a final TPN mixture contain, as a result of contamination in processing or because of inherent features of the constituents, some specific concentration of trace elements. This concentration can range from undetectable levels to amounts in excess of the recommended daily parenteral requirements. It appears important to determine how much of a specific trace element is present in a TPN mixture before parenteral recommendations and logical adjustments with additives can be made.

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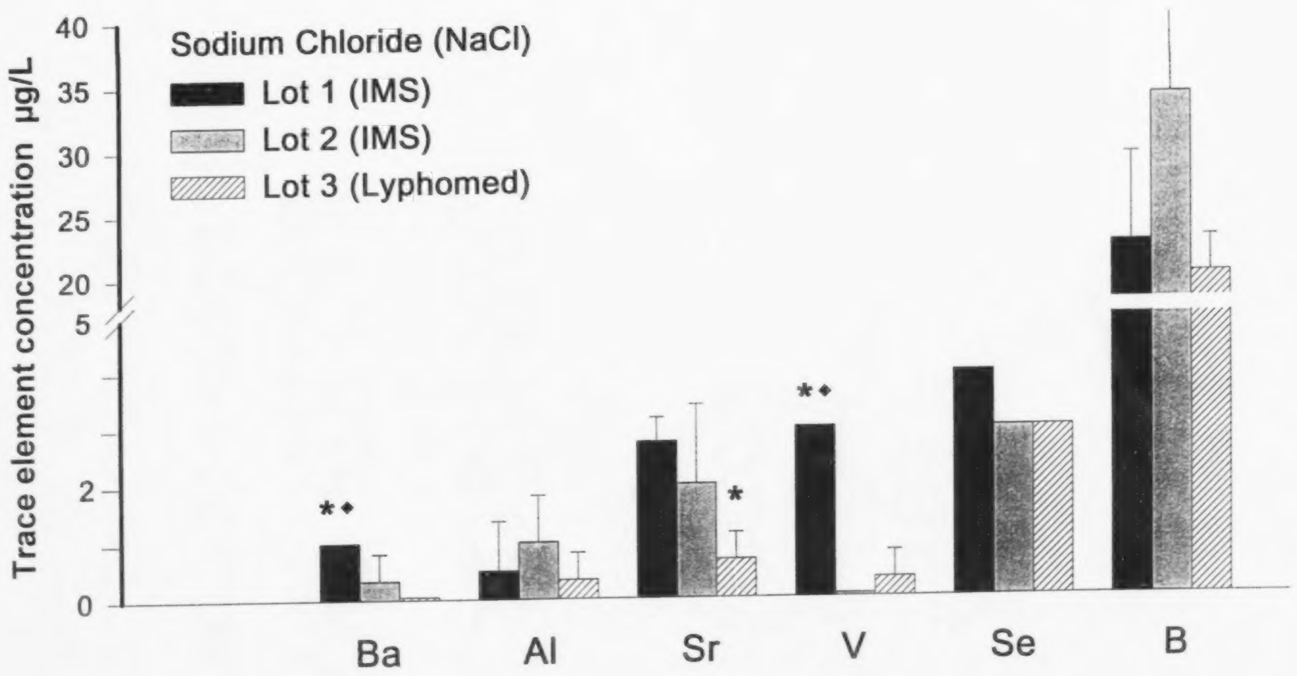

Figure 3-1. Concentration of trace element contaminants from the 4mmol/mL NaCl solution. Values represent the concentration of trace elements which would be present when the component was mixed to 1L of standard TPN solution outlined in Table 2. Two different manufacturers are represented: International Medication Systems (IMS), Mississauga, ON, Canada and Lyphomed, Markham, ON, Canada. Expected concentration of contaminants = 0 µg/L. [Ba]: Lot 1 (IMS) greater than Lots 2 (IMS) and 3 (Lyphomed), Lot 3 = 0µg/L; *◆$p<0.003$. [Sr]: Lot 3 (Lyphomed) less than Lot 1 (IMS); *$p<0.04$. [V]: Lot 1 (IMS) greater than Lots 2 (IMS) and 3 (Lyphomed), Lot 2 = 0 µg/L; *◆$p<0.0001$. Results expressed as mean ± s.d. (Lot 1 n=4 samples; Lots 2 and 3 n=3 samples. (Se n=1 sample per lot represented; no statistics performed.)

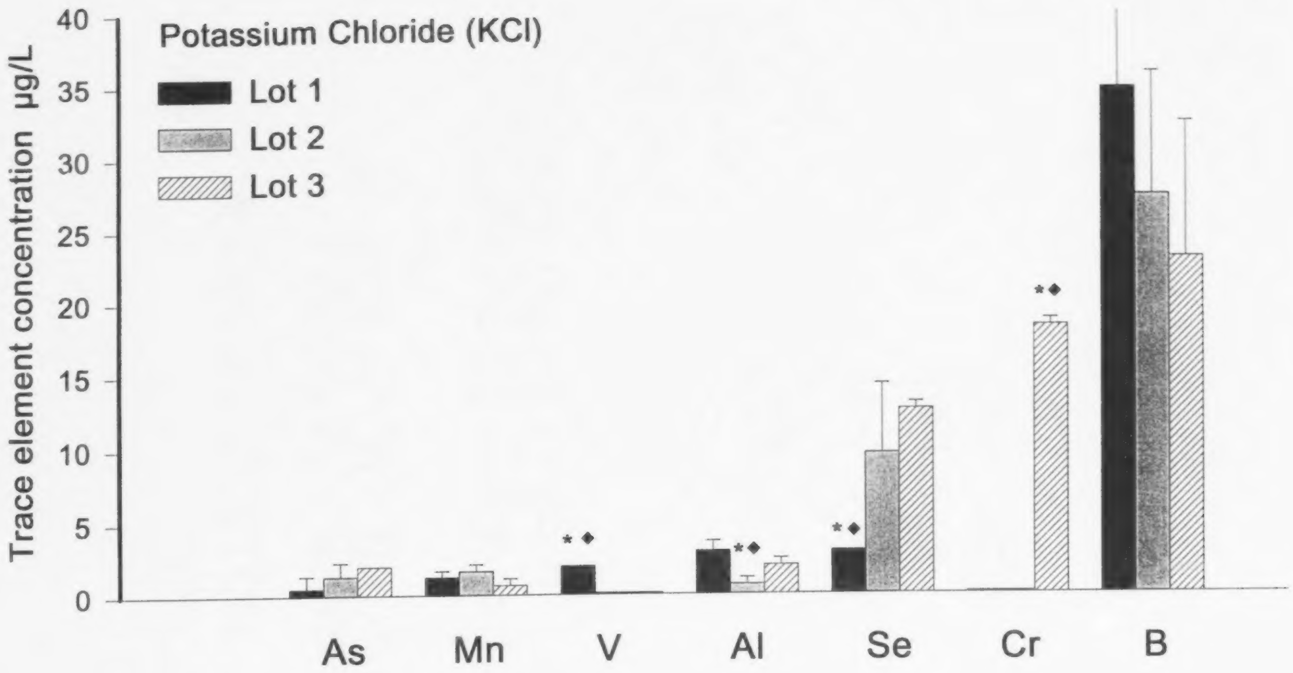

Figure 3-2. Concentration of trace element contaminants from the 2mmol KCl solution. Values represent the concentration of trace elements which would be present when the component was mixed to 1 L of standard TPN solution outlined in Table 2. Lots supplied by Abbott Laboratories, Montreal, PQ, Canada. Expected concentration of contaminants = 0. [V]: Lot 1 greater than Lots 2 and 3 (which=0µg/L); *◆$p<0.0001$. [Al]: Lot 2 less than Lots 1 and 3; *◆$p<0.005$. [Se]: Lot 1 less than Lots 2 and 3; *◆$p<0.004$. [Cr]: Lot 3 greater than Lots 1 and 2 (which=0µg/L); *◆$p<0.0001$. Results expressed as mean ± s.d. (Lot 1 n=4 samples; Lots 2 and 3 n=3 samples).

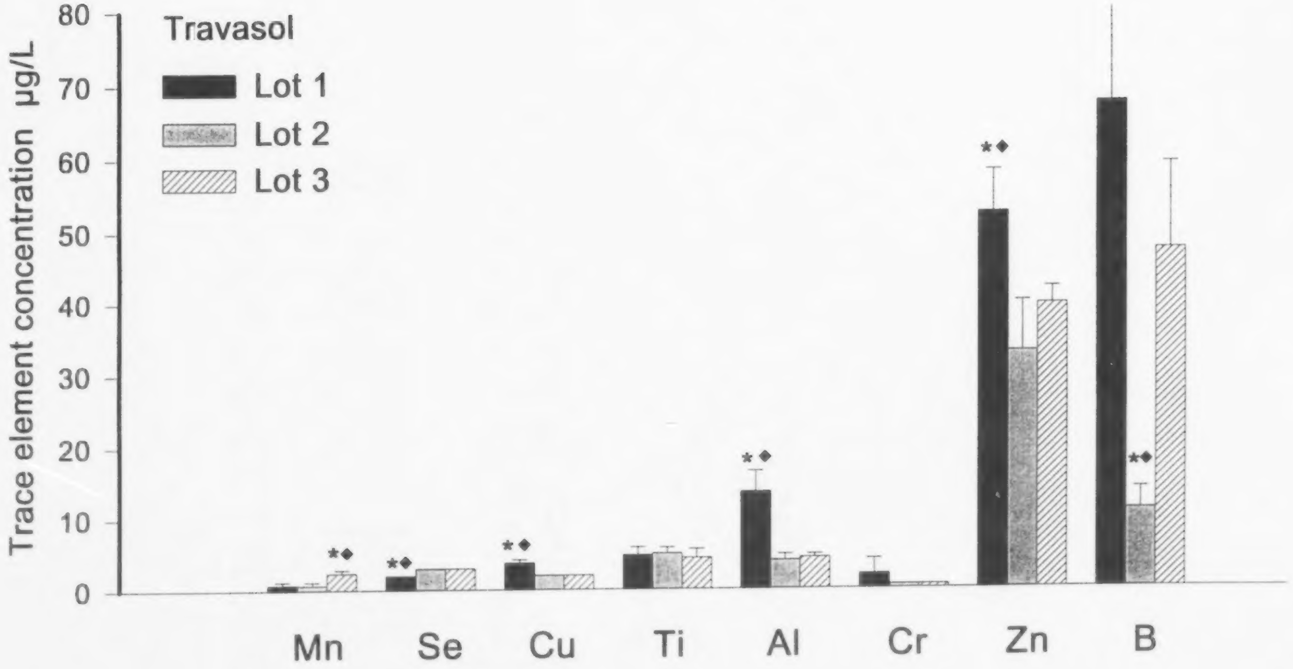

Figure 3-3. Concentration of trace element contaminants from the 10% Travasol solution. Values represent the concentration of trace elements which would be present when the component solution was mixed to 1L standard TPN solution outlined in Table 2. Lots supplied by Baxter Corporation, Toronto, ON, Canada. Expected concentration of contaminants = 0µg/L. [Mn]: Lot 3 greater than Lots 1 and 2; *♦$p<0.004$. [Se]: Lot 1 less than Lots 2 and 3; *♦$p<0.0001$. [Cu]: Lot 1 greater than Lots 2 and 3; *♦$p<0.0001$. [Al]: Lot 1 greater than Lots 2 and 3; *♦$p<0.0005$. [Cr]: Lot 1 based on value obtained by GFAAS (Quebec Centre for Toxicology); <2.0 µg/L; Lots 2 and 3 (which=0µg/L) (No statistics performed). [Zn]: Lot 1 greater than Lots 2 and 3; *♦$p<0.007$. [B]: Lot 2 less than Lots 1 and 3; *♦$p<0.05$. Results expressed as mean ± s.d. (Lot 1 n=4 samples; Lots 2 and 3 n=3 samples).

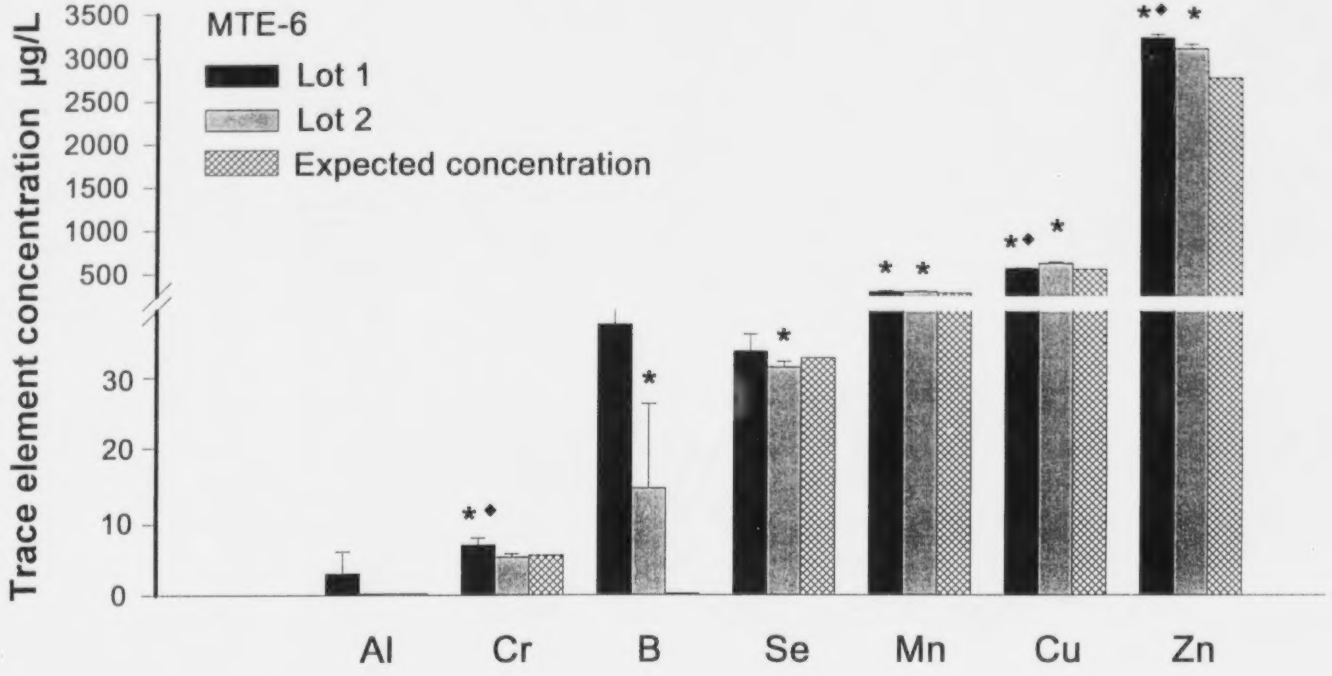

Figure 3-4. Concentration of trace elements and contaminants from the adult MTE-6 solution. Values represent the concentration of trace elements which would be present when the component was mixed to 1L standard TPN solution outlined in Table 2. Lots supplied by Lyphomed, Markham, ON, Canada. Expected concentration of trace elements represented by cross-hatched bars. Expected concentration represents labelled amounts from the manufacturer. Expected concentration of contaminants=0 µg/L. [Cr]: Lot 1 greater than Lot 2 (*$p<0.03$) and 'Expected' (◆$p<0.05$). [B]: Lot 2 greater than 'Expected'; *$p<0.04$. [Se]: Lot 2 less than 'Expected'; *$p<0.05$. [Mn]: Lots 1 and 2 greater than 'Expected'; *$p<0.04$, *$p<0.0003$, respectively. [Cu]: Lots 1 and 2 greater than 'Expected'; *$p<0.0001$, *$p<0.0004$, respectively. Lot 1 less than Lot 2; ◆$p<0.0001$. [Zn]: Lots 1 and 2 greater than 'Expected'; *$p<0.0001$, *$p<0.003$, respectively. Lot 1 greater than Lot 2; ◆$p<0.01$. Results expressed as mean ± s.d. (Lot 1 n=4 samples; Lots 2 and 3 n=3 samples).

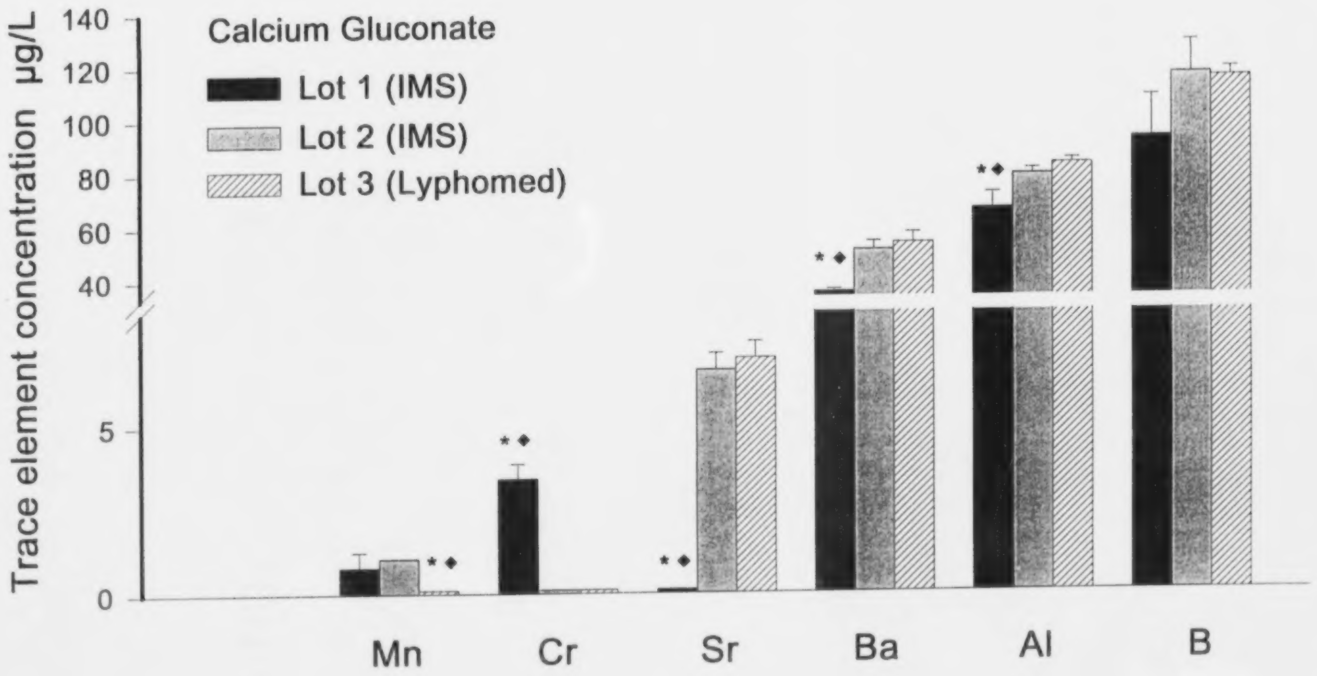

Figure 3-5. Concentration of trace element contaminants from the10% calcium gluconate solution. Values represent the concentration of trace elements which would be present when the component was mixed to 1L standard TPN solution outlined in Table 2. Two different manufacturers are represented: International Medication Systems (IMS), Mississauga, ON, Canada and Lyphomed, Markham, ON, Canada. Expected concentration of contaminants=0 µg/L. [Mn]: Lot 3 (0µg/L)(Lyphomed) less than Lots 1 and 2 (IMS); *•p<0.008. [Cr]: Lot 1 (IMS) greater than Lots 2 (IMS) and 3 (Lyphomed); (both of which=0 µg/L); *•p<0.0001. [Sr]: Lot 1 (IMS) (0µg/L) less than Lots 2 (IMS) and 3 (Lyphomed); *•p<0.0001. [Ba]: Lot 1 (IMS) less than Lots 2 (IMS) and 3 (Lyphomed); *•p<0.0001. [Al]: Lot 1 (IMS) less than Lots 2 (IMS) and 3 (Lyphomed); *•p<0.002. Results expressed as mean ± s.d. (Lot 1 n=4 samples; Lots 2 and 3 n=3 samples).

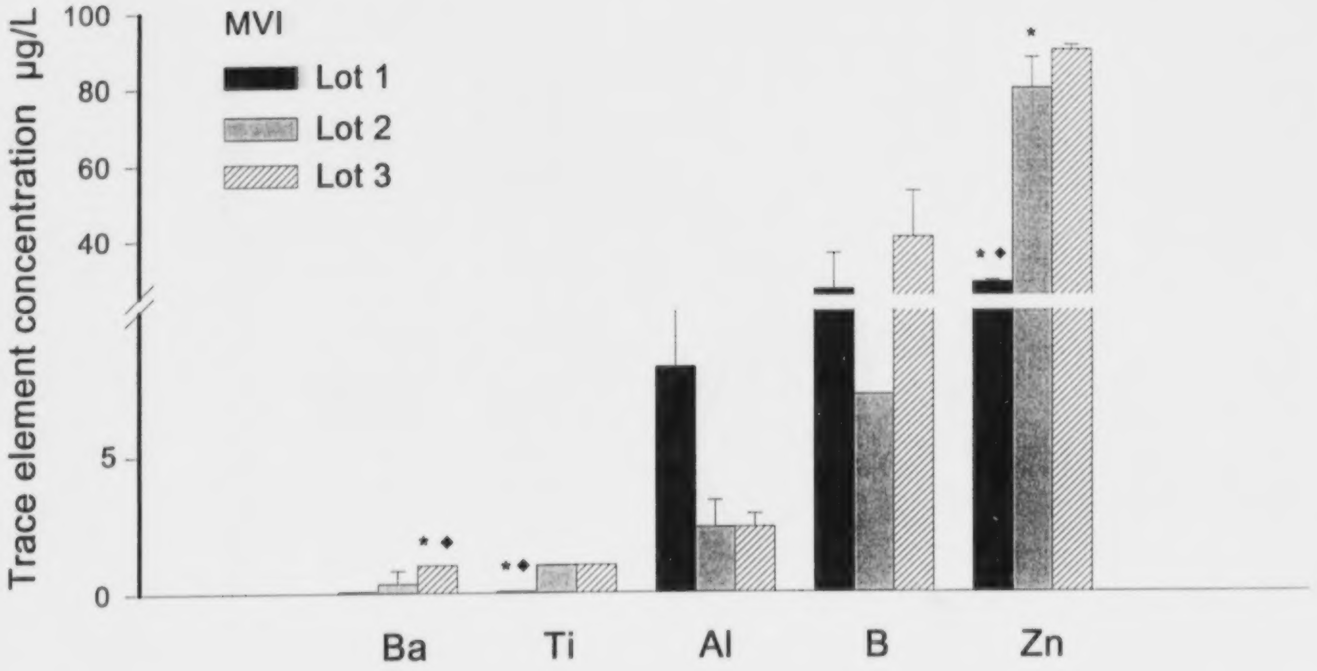

Figure 3-6. Concentration of trace element contaminants from the adult multivitamin (MVI) solution. Values represent the concentration of trace elements which would be present when the component was mixed to 1L of standard TPN solution outlined in Table 2. Lots supplied by Sabex, Boucherville, PQ, Canada. Expected concentration of contaminants = 0 µg/L. [Ba]: Lot 3 greater than Lots 1 (0µg/L) and 2; *•$p<0.004$. [Ti]: Lot 1 (0µg/L) less than Lots 2 and 3; *•$p<0.0001$. [Zn]: Lots 1and 2 less than Lot 3; *$p<0.0001$. Lot 1 less than Lot 2; •$p<0.0001$. Results expressed as mean ± s.d. (Lot 1 n=4 samples; Lots 2 and 3 n=3 samples).

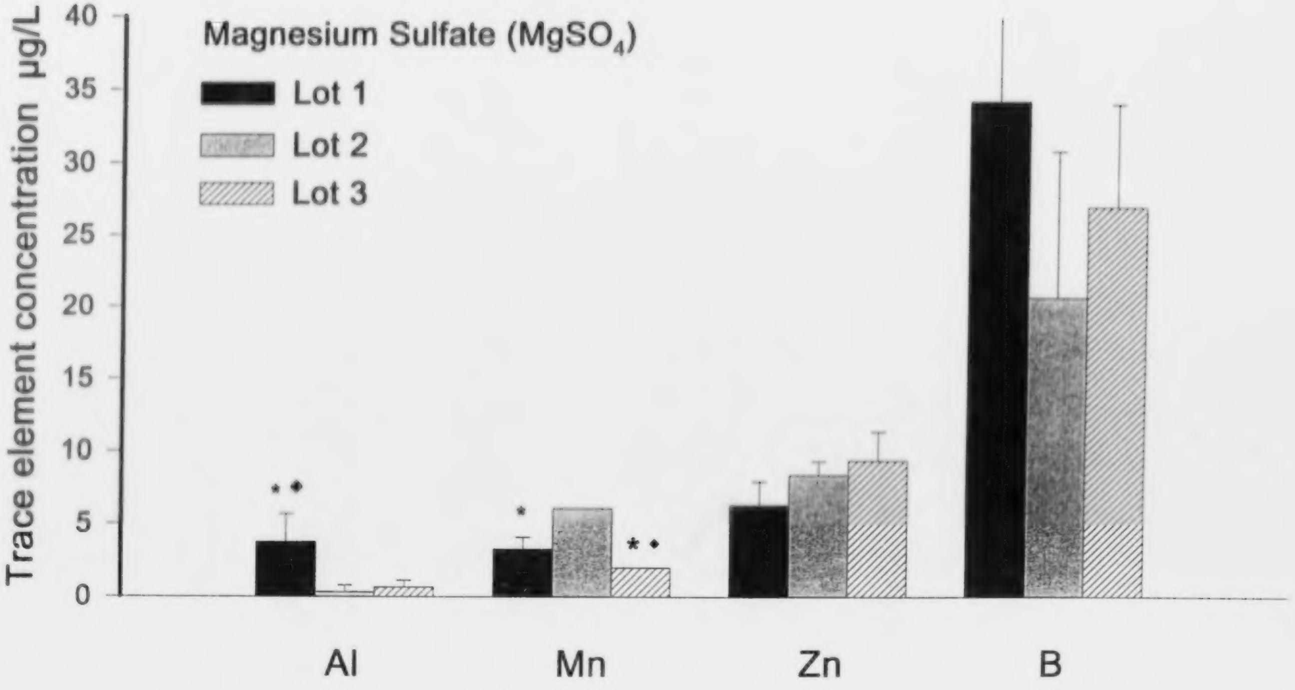

Figure 3-7. Concentration of trace element contaminants from the 50% $MgSO_4$ solution. Values represent the concentration of trace elements which would be present when the component was mixed to 1L standard TPN solution outlined in Table 2. Lots supplied by Sabex, Boucherville, PQ, Canada. Expected concentration of contaminants = 0μg/L. [Al]: Lot 1 greater than Lots 2 and 3; *•$p<0.02$. [Mn]: Lots 1 and 3 less than Lot 2; *$p<0.001$. Lot 3 less than Lot 1; •$p<0.001$. Results expressed as mean ± s.d. (Lot 1 n=4 samples; Lots 2 and 3 n=3 samples).

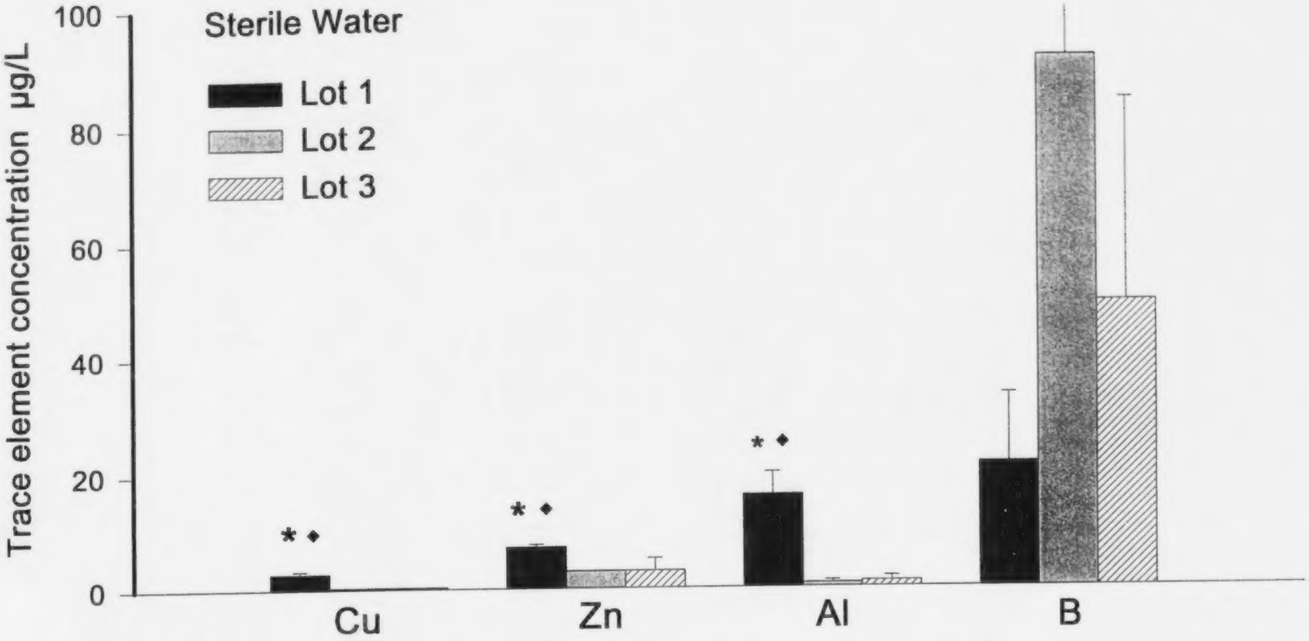

Figure 3-8. Concentration of trace element contaminants from the sterile water. Values represent the concentration of trace elements which would be present when the component was mixed to 1L of standard TPN solution outlined in Table 2. Lots supplied by Baxter Corporation, Toronto, ON, Canada. Expected concentration of contaminants = 0µg/L. [Cu]: Lot 1 greater than Lots 2 and 3 (both of which=0µg/L); *◆$p<0.0001$. [Zn]: Lot 1 greater than Lots 2 and 3; *◆$p<0.003$. [Al]: Lot 1 greater than Lots 2 and 3; *◆$p<0.0001$. Results expressed as mean ± s.d. (Lot 1 n=4 samples; Lots 2 and 3 n=3 samples).

Table 3-1. Lot numbers and manufacturers of components used to make a TPN solution

Component	Lot 1	Lot 2	Lot 3	Manufacturer
NaCl	FZ050G3[#]	FZ057L4[#]	140348[*]	IMS[#], Lyphomed[*]
KCl	87 803DM	93 446DM	95 616DM	Abbott
Travasol	AP426F7XS	AP432A9	AP437N3	Baxter
MTE-6	130104	140168	—	Lyphomed
Dextrose	C242933B	C264523	C278341	Baxter
Ca-gluconate	BB046L3[#]	BB047C4[#]	140169[*]	IMS[#], Lyphomed[*]
MVI	069404A	229407C	189411B	Sabex
$MgSO_4$	819305A	139410A	049408A	Sabex
Sterile water	AP431W0	AP448A0	AP451F8	Baxter

IMS = International Medication Systems, Limited; Mississauga, Ontario
Lyphomed; Markham, Ontario
Abbott Laboratories, Limited; Montreal, Quebec
Baxter Corporation; Toronto, Ontario
Sabex; Boucherville, Quebec

Table 3-2. Volume of components used to compose a one litre standard TPN solution

Solution	Volume (mL)
NaCl 4mM/mL	11.66
KCL 2mM	22.22
Travasol 10% without electrolytes	300.00
MTE-6 trace element concentrate[1]	0.56
Dextrose 70%	257.14
Ca-gluconate 10%	19.16
MVI adult multivitamin[2]	5.56
$MgSO_4$ 50%	2.18
Sterile water	381.52
Total	1000.00

[1] MTE-6 concentrate contains per mL: 5 mg Zn, 1 mg Cu, 0.5 mg Mn, 10 µg Cr, 60 µg Se, 75 µg I.

[2] MVI adult contains per 10 mL: vitamin C 1000 mg, vitamin A 10 000 IU, vitamin D 1000 IU, vitamin E (dl-alpha tocopheryl acetate) 10 IU, thiamine (as hydrochloride) 45 mg, riboflavin (as sodium phosphate) 10 mg, niacinamide 100 mg, pyridoxine (as hydrochloride) 12 mg, d-pantothenic acid (as d-panthenol) 26 mg, benzyl alcohol 9 mg.

Table 3-3. Trace elements examined with the "Spectrum-directed" ICP-MS screen

Li (Lithium)	Ni (Nickel)	Zr (Zirconium)	Te (Tellurium)	Gd (Gadolinium)	Re (Rhenium)
Be (Beryllium)	Cu (Copper)	Nb (Niobium)	I (Iodine)	Tb (Terbium)	Os (Osmium)
B (Boron)	Zn (Zinc)	Mo (Molybdenum)	Xe (Xenon)	Dy (Dyprosium)	Ir (Iridium)
Al (Aluminum)	Ga (Gallium)	Ru (Ruthenium)	Cs (Cesium)	Ho (Holmium)	Pt (Platinum)
Sc (Scandium)	Ge (Germanium)	Rh (Rhodium)	Ba (Barium)	Er (Erbium)	Au (Gold)
Ti (Titanium)	As (Arsenic)	Pd (Palladium)	La (Lanthanum)	Tm (Thulium)	Hg (Mercury)
V (Vanadium)	Se (Selenium)	Ag (Silver)	Ce (Cerium)	Yb (Ytterbium)	Tl (Thallium)
Cr (Chromium)	Kr (Krypton)	Cd (Cadmium)	Pr (Praseodymium)	Lu (Lutetium)	Pb (Lead)
Mn (Manganese)	Rb (Rubidium)	In (Indium)	Nd (Neodymium)	Hf (Hafnium)	Bi (Bismuth)
Fe (Iron)	Sr (Strontium)	Sn (Tin)	Sm (Samarium)	Ta (Tantalum)	Th (Thorium)
Co (Cobalt)	Y (Yttrium)	Sb (Antimony)	Eu (Europium)	W (Tungsten)	U (Uranium)

Table 3-4a. **Percent contribution of Lot 1 components to the total trace element concentration in a standard TPN solution**

Component	Zn	Cu	Mn	Cr	Se	B	Al	Ti	Ba	V	As	Sr
NaCl	—	—	—	—	9	3	0	—	3	60	—	100
KCl	—	—	0.4	—	7	4	1	—	—	40	100	—
Travasol	1.5	0.7	0.3	9*	5	8	6	95	—	—	—	—
MTE-6	96.6	98.0	98.0	56	78	4	1	—	—	—	—	—
Dextrose	0.5	0.8	—	9*	—	62	47	—	—	—	—	—
Ca-gluconate	—	—	0.3	26	—	10	31	—	97	—	—	—
MVI adult	0.9	—	—	—	—	3	4	5	—	—	—	—
$MgSO_4$	0.2	—	1	—	—	4	2	—	—	—	—	—
Sterile water	0.3	0.5	—	—	—	2	8	—	—	—	—	—
Total	100	100	100	100	100	100	100	100	100	100	100	100

*Based on values obtained from GFAAS analysis, Quebec Centre for Toxicology

Table 3-4b. Continued. **Percent contribution of Lot 2 components to the total trace element concentration in a standard TPN solution**

Component	Zn	Cu	Mn	Cr	Se	B	Al	Ti	Ba	V	As	Sr
NaCl	—	—	—	—	6.3	5.1	1.1	—	0.6	—	—	24
KCl	—	—	0.6	—	20.3	4.0	0.7	—	—	—	100	—
Travasol	1.0	0.3	0.2	—	6.3	1.6	4.2	26.3	—	—	—	—
MTE-6	95.6	99.7	96.9	100	67.1	2.3	—	—	—	—	—	—
Dextrose	0.6	—	—	—	—	52	7.1	68.4	—	—	—	—
Ca-gluconate	—	—	0.3	—	—	17.2	83.4	—	98.8	—	—	76
MVI adult	2.5	—	—	—	—	1.0	2.4	5.3	0.6	—	—	—
$MgSO_4$	0.2	—	2.0	—	—	3.1	0.3	—	—	—	—	—
Sterile water	0.1	—	—	—	—	13.7	0.8	—	—	—	—	—
Total	100	100	100	100	100	100	100	100	100	0	100	100

Table 3-4c. Continued. Percent contribution of Lot 3 components to the total trace element concentration in a standard TPN solution

Component	Zn	Cu	Mn	Cr	Se	B	Al	Ti	Ba	V	As	Sr
NaCl	—	—	—	—	16	3.8	0.3	—	—	100	—	8.2
KCl	—	—	12.3	100	68	4.4	1.9	—	—	—	100	—
Travasol	25.6	100	46.9	—	16	8.9	4.0	28.1	—	—	—	—
MTE-6*	—	—	—	—	—	—	—	—	—	—	—	—
Dextrose	8.6	—	—	—	—	38.7	12.4	65.4	—	—	—	—
Ca-gluconate	—	—	—	—	—	21.8	77.6	—	98.1	—	—	91.8
MVI adult	57.9	—	—	—	—	7.8	2.2	6.5	1.9	—	—	—
$MgSO_4$	6	—	40.8	—	—	5.1	0.7	—	—	—	—	—
Sterile water	1.9	—	—	—	—	9.5	0.9	—	—	—	—	—
Total	100	100	100	100	100	100	100	100	100	0	100	100

*Lot 3 no MTE-6, therefore, no added Zn, Cu, Mn, Cr, and Se. Values expressed as percentages based on the presence of contaminants only.

Table 3-5. Concentration of trace elements from TPN solutions composed of Lot 1 and 2 components relative to the expected concentration in a standard TPN solution

Element	Expected[1] concentration in 2L TPN solution (μg/L)	Calculated amount in 2L TPN from Lot 1 (μg/L)	Percent difference from expected	Calculated amount in 2L TPN from Lot 2 (μg/L)	Percent difference from expected	Average amount absorbed from daily oral diet (μg)[4]
Zn	5560	6682	20 (+)[2]	6508	17 (+)	4000
Cu	1120	1202	7 (+)	1264	13 (+)	640 to 840
Mn	560	598	7 (+)	610	9 (+)	165 to 209
Cr[3]	11	26	136 (+)	11	0	80 to 160
Se	67	88	31 (+)	96	43 (+)	0.7 to 3.5
B	0	1796	> 100 (+)	1346	> 100 (+)	900 to 2700
Al	0	428	> 100 (+)	176	> 100 (+)	200
Ti	0	10	> 100 (+)	40	> 100 (+)	2 to 20
Ba	0	72	> 100 (+)	104	> 100 (+)	36 to 54
V	0	11	> 100 (+)	0	0	0.06 to 0.165
As	0	1	> 100 (+)	3	> 100 (+)	18 to 90[5]
Sr	0	6	> 100 (+)	16	> 100 (+)	89 to 342

[1] Concentration of trace elements present in 1.2 mL MTE-6, based on AMA daily intravenous trace element recommendations for TPN delivery.

[2] (+) denotes increase over expected level.

[3] Calculated amounts of Cr from dextrose and travasol in Lot 1 components based on measurements by graphite furnace atomic absorption spectrometry. Lot 2 based on corrected value with ICP-MS.

[4] Based on values from Chapter 5, Table 5-3.

[5] Reported daily intake 20 to 100 μg/day from Health and Welfare Canada. Nutrition Recommendations. Report of the Scientific Review Committee. Ottawa: Canadian Government Publishing Centre, 1990. Absorption factor 90% from Iffland R. Arsenic. In: Seiler HG, Sigel H and Sigel A, eds. Handbook on Metals in Clinical and Analytical Chemistry.\ New York: Marcel Dekker, 1994: 237-253. Daily intake x absorption factor (20 to 100 μg x 0.90) = 18 to 90 μg.

Table 3-6. Summary of previously reported trace element contamination of TPN components.

Component	Zn µg/mL (µg/L TPN solution)[*]	Cu µg/mL (µg/L TPN solution)	Mn µg/mL (µg/L TPN solution)	Cr µg/mL (µg/L TPN solution)	Se µg/mL (µg/L TPN solution)	B µg/mL (µg/L TPN solution)	Al µg/mL (µg/L TPN solution)	V µg/mL (µg/L TPN solution)
NaCl	0.015 to 0.428[1,2] (0.14 to 6.63)	0.175[2] (2.71)	ND[#] to 0.023[3] (0 to 0.27)	ND to <0.002[4,5] (0 to <0.02)	<0.03[6] (<0.3)	ND	<0.005 to0.006[8,9] (<0.06 to 0.07)	0 to 0.14[7] (0 to 1.63)
KCl	0.115[2] (2.6)	0.160[2] (3.6)	0.006 to 0.007[3] (0.13 to 0.16)	0.001 to 0.009[5] (0.02 to 0.2)	<0.03[6] (<0.7)	0 to 0.02[7] (0 to 0.4)	0.005 to 0.017[9] (<0.1 to 0.4)	0 to 0.1[7] (0 to 2.22)
Amino Acid:								
FreAmine II 8.5%	0.56 to 4.0[2,10,11] (168 to 1200)	0.009 to 0.085[2,10,11] (2.7 to 25.5)	0.59 to 0.172[11] (17.7 to 51.6)	0.0022 to 0.0024[10] (0.66 to 0.72)	—	—	—	—
8.5% amino acids[@]	0.18 to 0.271[4] (54 to 81)	0.032 to 0.065[4] (9.6 to 19.5)	ND[4]	ND[4]	—	—	—	—
Travasol 10%	—	—	0.001[3] (0.4)	0.001 to 0.002[5] (0.3 to 0.6)	—	ND	0.006 to 0.026[9,12] (1.8 to 7.8)	0 to 0.09[7] (0 to 27)
Trace Elements	—	—	—	9.3 to 11.3[5] (5.2 to 6.3)	—	—	0.136[9] (0.08)	—
Dextrose 70%	ND[2]	0.092[2] (23.7)	0.002[3] (0.5)	—	0.33[6] (84.9)	0.02 to 0.11[7] (5.6 to 28.6)	—	ND[7]
Ca-gluconate	—	—	0.076 to 0.18[3] (1.4 to 3.2)	0.002 to 0.0084[5] (0.04 to 0.2)	0.033 to 0.613[6] (0.6 to 11.7)	2.03 to 2.54[7] (44.1 to 48.7)	1.095 to 5.57[8,9] (21 to 106.6)	—

Component	Zn µg/mL (µg/L TPN solution)[*]	Cu µg/mL (µg/L TPN solution)	Mn µg/mL (µg/L TPN solution)	Cr µg/mL (µg/L TPN solution)	Se µg/mL (µg/L TPN solution)	B µg/mL (µg/L TPN solution)	Al µg/mL (µg/L TPN solution)	V µg/mL (µg/L TPN solution)
MVI	2.30[2] (12.8)	0.078[2] (0.4)	0.004 to 0.063[3] (0.02 to 0.4)	0.009 to 0.01[5] (0.05 to 0.06)	0.042 to 0.290[6] (0.2 to 1.6)	0.02 to 0.721[7] (0.1 to 4)	0.012 to 1.02[9] (0.07 to 5.7)	ND
MgSO$_4$	0.110[2] (0.23)	0.172[2] (0.37)	0.017 to 0.225[3] (0.04 to 0.5)	0.048 to 0.076[5] (0.1 to 0.2)	<0.03[6] (<0.07)	0.368 to 2.08[7] (0.8 to 4.5)	<0.005 to 0.005[9] (<0.01 to 0.01)	0.62 to0.85[7] (1.3 to 1.9)
Sterile water	ND to 0.1[1,2] (0 to38.2)	0.078[2] (29.8)	ND[3]	<0.002[5] (<2.0)	————	0 to 0.13[7] (0 to 49.5)	<0.005[9] (<2.0)	0.015 to 0.05[7] (5.6 to 19.4)

[*] µg/mL represents concentration of trace element per mL of the component solution. (µg/L TPN solution) denotes the concentration of trace element present in 1 L of standard TPN solution as reported in Table 3-2.

[#] ND = Not detected

[@] 8.5% Amino acid unspecified

[1] Reference [1]
[2] Reference [2]
[3] Reference [7]
[4] Reference [6]
[5] Reference [8]
[6] Reference [9]
[7] Reference [12]
[8] Reference [11]
[9] Reference [10]
[10] Reference [4]
[11] Reference [3]
[12] Reference [34]

CHAPTER 4

COMPOUNDING EQUIPMENT CONTRIBUTES TRACE ELEMENT CONTAMINANTS TO SOLUTIONS

SPECIFIC AIM

The specific aim of this experimental section was to determine whether or not additional trace elements were contaminating solutions as a result of exposure to compounding devices.

INTRODUCTION

It is well known that the components used to formulate TPN solutions are contaminated with trace elements such as Zn, Cu, Cr, and Mn [1], as well as Mo, Ni, V, Cd, Al, and B [2]. When compounded into a TPN solution, this contamination can result in the inadvertent delivery of excess trace elements to patients, who in turn, may accumulate toxic levels. For most trace elements, the intravenously delivered concentrations necessary to produce toxicity symptoms are not yet known. The literature published to date has not made clear whether or not the pharmaceutical compounding systems used in health care facilities and industry are also linked to this trace element contamination.

In the past, TPN compounding was performed manually using a hyperalimentation transfer set comprised of a needle, syringe, filter, and a length of plastic tubing. The addition of individual solution components to the TPN container was often time-consuming and tedious, especially if many additives were required per unit and a large number of units were to be prepared. At present, however, manual compounding is rare. It is typically reserved for special small volume (ie, <200 mL) parenteral solutions, such as those prepared for pediatric ICU patients. Most hospitals prepare daily TPN orders using automatic compounding devices. These devices employ a computer program which calculates the appropriate volume of each component for a prescribed TPN solution. The individual amounts are then pumped from stock containers through a number of plastic lines into a plastic TPN bag.

A variety of materials come into contact with the TPN solution during manual and automatic compounding procedures. These materials may serve as sources of trace element contamination. Though plastic is generally cleaner or less "contaminated" with trace elements than other materials, such as glass, it is a complex material and does contain modest levels of certain trace metals [3]. These trace elements may be leeched from the plastic into the TPN solution [4]. Additionally, any adhering trace element residues may also be eluted from plastic in contact with a solution.

Disposable syringes, like those used for manual compounding, are often made of polyethylene or polypropylene. In one summary of the average element content of various materials, polyethylene was shown to contain 22 different elements (total concentration: 654 mg/L); the major constituents were Ca, Zn, and Si [3]. Similarly, polypropylene was shown to contain 21 elements (total concentration: 519 mg/L) and the major constituents were Cl, Mg, and Ca [3]. The tubing used in manual and automatic compounding devices, in addition to the plastic TPN bag itself, is made of transparent polyvinyl chloride. Polyvinyl chloride (tubing) has been shown to contain approximately nine trace elements (total concentration: 280 mg/L), the major constituents of which are Fe, Zn, and Sb [3]. Whether or not these elements leech from tubing or plastic bags during TPN compounding procedures does not appear to be known.

The needle and syringe system used during the manual compounding process may also serve as a source of trace element contamination. Significant contamination by Pb and Mn can originate in the rubber seal on the plunger of syringes [5]. Chromium, Fe, Ni, and Zn contamination might also result from contact with this rubber, the manufacture of which requires a Zn-based catalyst (Zn oxide or Zn diethyl dithiocarbamate) [5]. Needles may also be the source of trace element contaminants because of their release of metal ions; for instance Ni and Mo are both common elements in steel needles [6]. Needles composed of various metal alloys (Al, Co, Cr, Mn, Mo, Ni, V) are also potential sources of contamination [5]. Experimental observations have confirmed that Ni is eluted from these needles. After four passages of physiological saline (original Ni level of 10 μg/L) through a needle, a Ni concentration of approximately 45 μg/L was reported; after 12 passages, concentrations varied from 104 to 253 μg/L [7]. During the process of manual TPN compounding with a hyperalimentation transfer set, significant contamination of the TPN with eluted trace elements might conceivably occur.

Given the nature of the materials which come into contact with the TPN components during compounding, it is important to determine whether or not trace element contamination occurs during the TPN compounding procedure. The following experiment, therefore, examined the possibility that inadvertent trace element contamination of TPN solutions might result from the use of automated and manual compounding systems. In addition, the study sought to identify the particular sources of the contamination within the compounding procedure.

MATERIALS AND METHODS

Supplies

Bags of sterile water (Baxter, Toronto, Canada; Lot AP439N0), TPN bags automatically compounded (Baxa MicroMacro 23 Compounder with IEEE 488 Interface) with 500 mL sterile water (Baxter, Toronto, Canada; Lot AP439N0), empty 500 mL TPN bags (Exacta-Med Container, Baxa, Englewood, CO), and disposable Cornwall Syringe sets (Becton-Dickenson, Rutherford, N.J.; No. 5224) for manual compounding were purchased from the University of Alberta Hospitals Pharmacy, Walter Mackenzie Health Sciences Centre, Edmonton, Canada.

Experimental Groups

Four experimental groups were investigated in order to delineate the source(s) from which trace element contamination could be introduced during the TPN compounding procedure: 1) sterile water as received from the manufacturer, 2) TPN bags manually compounded with sterile water, 3) TPN bags automatically compounded with sterile water, and 4) sterile water manually compounded into sample vials (ie, bypassing the TPN bag).

Sample Collection

Under a laminar air flow hood equipped with a HEPA-filter, and using non-powdered gloves as a precaution against extraneous trace element contamination, 30 mL samples of sterile water were poured from the delivery port of each of the bags from the manufacturer and transferred to trace element-free high density polypropylene vials (Sarstedt, St. Laurent, Quebec). Empty TPN bags were manually compounded with 500 mL of this sterile water using a disposable Cornwall Syringe system equipped with a 60 mL syringe and 19 gauge needle

(Becton-Dickinson, Rutherford, NJ). The manually and automatically compounded TPN bags of sterile water were stored in the usual manner at 4°C. Approximately sixteen hours later, 30 mL samples were poured from the delivery port of each of the manually and automatically compounded TPN bags of sterile water into trace element-free high density polypropylene vials. To obtain samples for the fourth experimental group, a sterile water bag from the manufacturer was punctured with a Cornwall syringe set connected to a 60 mL syringe and 19 gauge needle. Instead of being manually transferred to an empty TPN bag, the samples of sterile water were ejected directly into trace element-free high density polypropylene sample vials, thereby bypassing any potential contamination represented by the TPN bag. This direct injection allowed us to determine whether the TPN bag was a source of trace element contamination.

Trace Element Analysis

Samples were analyzed for trace elements using ICP-MS (Perkin Elmer Sciex Elan 5000A ICP-MS System, Perkin Elmer Inc. Toronto, ON). Analyses were performed in two parts. First, a "Spectrum-directed" analysis for 66 trace elements (excluded were major elements) was performed employing the Total Quant Mode (scanning from atomic mass 3[Li] to 92[U]) to screen for any exisiting contaminants (Table 4-1). One sample was taken from each of five bags of sterile water from the manufacturer, TPN bags manually compounded with sterile water, and TPN bags automatically compounded with sterile water for the "Spectrum-directed" screen. Based upon the results of the screen, any trace elements appearing at a level ≥ 1μg/L were considered to be present at a level sufficient to warrant specific "Element-directed" quantitative analysis employing the Quantitative Mode of the ICP-MS. Approximately seven days later the "Element-directed" portion of the analysis was performed on four samples from each of four bags of sterile water from the manufacturer, TPN bags manually compounded with sterile water, TPN bags automatically compounded with sterile water and sterile water manually compounded directly into the sample vials (ie, bypassing the TPN bag).

Data Analysis

The results are reported as means ± SEM. An analysis of variance and the Student-Newman-Keuls test (where appropriate) were employed to identify significant differences using the SigmaStat statistical software package (Jandel Scientific, San Rafel, CA). A probability value of $p<0.05$ was considered to be significant.

RESULTS

Element-directed quantitative analysis revealed the presence of only three trace element contaminants, Zn, B, and Al, in each of the experimental groups. Zinc, B and Al were contaminants of the sterile water obtained from the manufacturer. Additional Zn contamination occured as a consequence of the TPN bag and the compounding process.

Sterile water from the maunfacturer. Element-directed quantitative analysis revealed that small background amounts of Zn (13.9 ± 0.7 μg/L), B (1.6 ± 0.2 μg/L), and Al (1.3 ± 0.2 μg/L) were present in the sterile water obtained from the manufacturer. (Note: this sterile water was of a different lot number than those tested in Chapter 3)

TPN bags manually compounded with sterile water. The concentration of Zn in the manually compounded sterile water was significantly increased over that found in the sterile water bags from the manufacturer(34.2 ± 3.4 μg/L vs 13.9 ± 0.7 μg/L, respectively; <0.001) (Figure 4-1).

TPN bags automatically compounded with sterile water. The concentration of Zn in the automatically compounded sterile water was also significantly increased over that found in the sterile water bags from the manufacturer (36.6 ± 1.2 μg/L vs 13.9 ± 0.7 μg/L, respectively; $p<0.0001$). There was no difference, however, in the Zn contamination when the manual and automated compounding procedures were compared (34.2 ± 3.4 μg/L vs 36.6 ± 1.2 μg/L, respectively) (Figure 4-1).

Sterile water manually compounded directly into sample vials. This experimental group was included to determine whether or not the trace element contamination found after compounding was derived from the TPN bag, compounding components, or a combination of the two variables. Sterile water manually compounded directly into sample vials (ie, no TPN bag) demonstrated a Zn contamination of 26.3 ± 1.3 μg/L (which included the 13.9 μg/L from the sterile water). The concentrations of B and Al in the manually compounded sterile water not in contact with a TPN bag did not show any significant difference when compared to the concentrations found in the other experimental groups (Figure 4-2).

Calculation of Zn contamination from different sources. Since sterile water (manually or automatically) compounded into a TPN bag demonstrated a mean Zn contamination of 35.4 μg/L, the difference (35.4 - 26.3) of 9.1μg/L, represented that coming from the TPN bag. In order to determine the amount of Zn contamination coming from the compounding equipment, we used the following formula: Total [Zn] (35.4μg/L) - [Zn] Sterile water (13.9 μg/L) - [Zn] TPNbag (9.1 μg/L)=[Zn] Compounding. The amount of Zn contamination from the compounding equipment was thus 12.4μg/L.

DISCUSSION

Of the 66 trace elements originally scanned, only one was found to be a contaminant occurring as a result of TPN compounding. The entire process (whether automated or manual) of compounding sterile water into TPN bags added a total of 35.4 μg/L of Zn as a contaminant. The compounding equipment contributed about one third of this total (12.4 μg/L). In both types of compounding, the various TPN component solutions came into contact with polyvinyl chloride tubing. There is evidence that polyvinyl chloride tubing like that used in automated and manual compounding systems is contaminated with modest amounts of Zn during manufacturing [3]. The contamination observed in this experiment may have been due to a small amount of Zn being leeched or eluted from the tubing. The TPN bag was also shown to independently contribute 9 .1μg/L of Zn to the sterile water. Since the TPN bag was made of transparent polyvinyl chloride, it can be surmised that this Zn contamination was also leeched or eluted from the plastic material.

Trace amounts of Zn were found in the sterile water from the manufacturer (ie, 13.9 μg/L). This amount represents Zn which was inadvertently added at the point of manufacture. Zinc is a ubiquitous contaminant of rubber and glass, as well as of water and many chemicals, even those of the highest analytical grade [8]. The Zn in the sterile water could have been leeched from the polyvinyl chloride bag in which it was stored. It also could have originated in the water purification process. A number of materials, most notably glass and stainless steel, are used in the manufacture of sterile water. The borosilicate glass frequently used by the pharmaceutical industry, for example, often contains contaminants such as Zn and other elements along with the B and Al oxides deliberately incorporated into the silicate [9]. When water is sterilized, these trace elements may be leeched from the surfaces of glass and steel containers [9].

It thus appears that a small amount of Zn is added at each point in the compounding procedure; the sterile water, the TPN bag, and the implements used in the compounding procedure all contributed Zn contaminants. However, the amount of Zn added by the TPN compounding procedure is inconsequential when compared to the amount needed to satisfy physiological requirements. The American Medical Association recommendations for the addition of Zn to parenteral solutions prepared for stable adults are 2.5 to 4.0 mg/day [10]. The Zn contamination seen in this study represents approximately 1% of this daily requirement. The amount of Zn added during the TPN compounding process also appears insignificant when compared to the amounts contributed by contaminated TPN component solutions, as demonstrated in Chapter 3 of the thesis.

Negligible amounts of B and Al were found in each of the groups studied. Compounding sterile water into the TPN bag did not alter the initial B and Al concentrations. These elements were present in the bags of sterile water as received from the manufacturer, as discussed above. The levels of Al and B contamination observed in this experiment are also insignificant in comparison to the contamination levels originating in TPN components themselves, as reported in Chapter 3 of the thesis.

Boron and Al appear in TPN solutions strictly as contaminants; they are not added as part of regular TPN regimens. As with a number of other trace elements, a paucity of knowledge regarding physiological effects and possible human requirements exist. This lack of factual knowledge, in turn, makes it difficult for clinicians and researchers to assess the consequences of the presence of these trace elements in TPN solutions.

The low levels of trace element contamination observed in this series of experiments disagree with the greater levels reported to be leeched from the surfaces of rubber, plastic, and metal components in the studies of the past. This can be attributed to a number of factors. Firstly, the pharmaceutical manufacturing industry has taken steps to reduce the leeching of trace elements from rubber components in contact with parenteral solutions. Many rubber products are now coated with protective films (eg, Teflon) to reduce the problems of surface/product interaction [9]. This technology has presumably been imparted to the rubber seals on the plungers of present-day syringes. Secondly, silicone-coated needles have become available for smooth injection. The silicone seal likely acts as a protective barrier against the leeching of trace elements upon contact with various solutions. The needles used in this study were of the siliconized type.

The low levels of trace elements (ie, Zn) leeched from the plastic components in this study may have resulted from the limited contact time with the sterile water. During automatic compounding sterile water was in contact with polyvinyl chloride tubing for less than one minute, and during manual compounding for less than five minutes. The low level of Zn contamination observed is likely more attributable to the elution of Zn residues from the surface of the new plastic components. The minimal storage time (sixteen hours) of sterile water in the polyvinyl chloride TPN bag at reduced temperature also provided less of an opportunity for leeching to occur. Since plastic is such a highly variable material as a result of its different additives and manufacturing processes, the type of polyvinyl chloride bags and tubing used in this experiment may have innately contained less trace element contaminants than in products tested previously.

In this study, sterile water was passed through compounding components with little accumulated trace element contamination. It is conceivable that the passage of different parenteral solutions (eg, NaCl, $MgSO_4$) through the same compounding procedure would be capable of leeching other trace elements from the rubber, plastic, and metal components. However, the extremely short contact time (< 15 seconds) of each of the solution components with the plastic tubing make such an observation unlikely.

In conclusion, compounding, either manually or via an automated system, contributes minimal amounts of trace element contaminants to the TPN solutions received by patients. Although the sources of trace element contamination of TPN solutions remains to be determined, it is likely that much results from the use of component solutions contaminated with trace elements. Whether or not the TPN delivery system, with its plastic infusion lines and filter systems, further contributes to or reduces this trace element contamination, provides an area for future investigation. Chapter 5 explores this issue.

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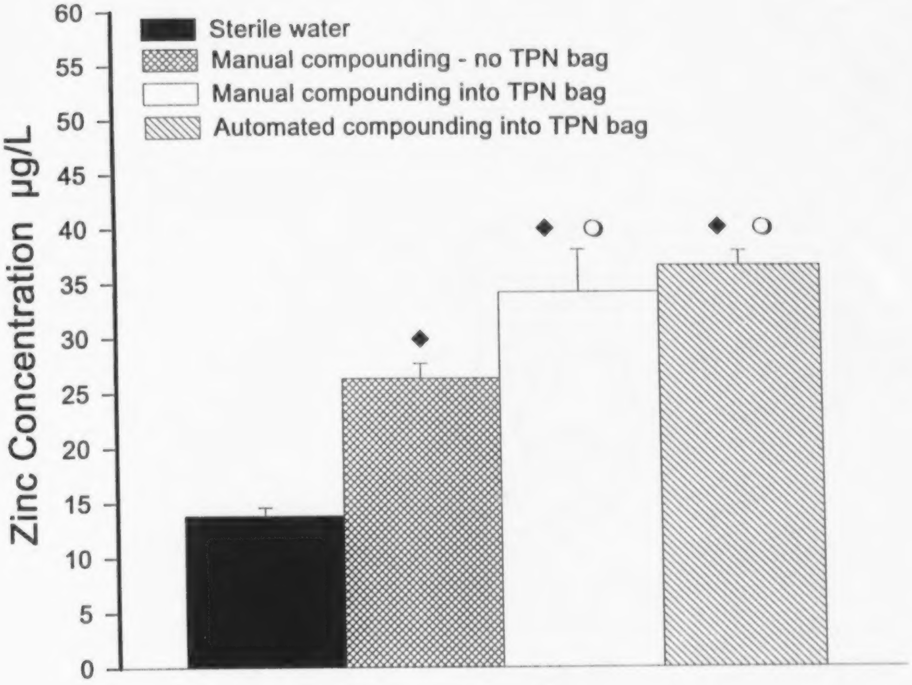

Figure 4-1. The concentration of Zn present as a contaminant in each of the experimental groups. Data represents means ± SEM of 4 samples from each of 4 TPN bags compounded with sterile water or bags of sterile water from the manufacturer. Sterile water compounded into TPN bags or directly into sample vials showed significantly greater contamination with Zn compared to sterile water from the manufacturer; •$p<0.05$. The compounding (automatic or manual) of sterile water into TPN bags added a significantly greater quantity of Zn to the samples compared to the compounding of sterile water directly into sample vials; ○$p<0.05$. This indicated that a portion of Zn contamination was derived from the TPN bag itself.

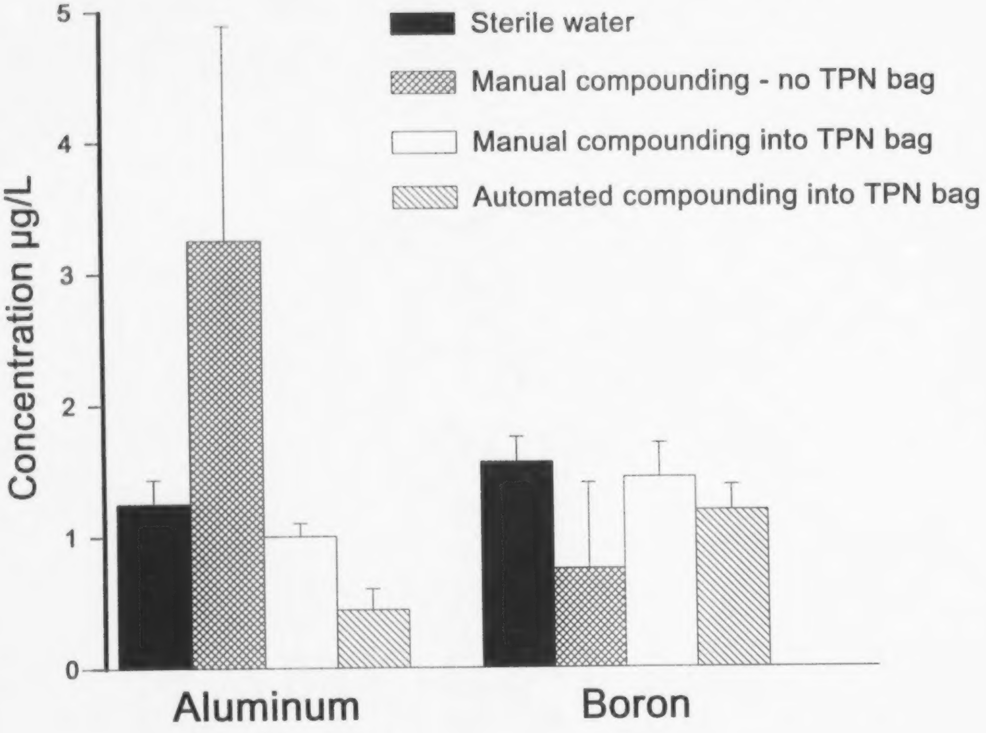

Figure 4-2. The concentration of Al and B present as contaminants in each of the experimental groups. Data represent means ± SEM of 4 samples from each of 4 TPN bags compounded with sterile water or bags of sterile water from the manufacturer. No significant changes in the original Al or B concentration of the sterile water from the manufacturer were apparent after compounding with the three procedures.

Table 4-1. Trace elements examined with the “Spectrum-directed” ICP-MS screen

Li (Lithium)	Ni (Nickel)	Zr (Zirconium)	Te (Tellurium)	Gd (Gadolinium)	Re (Rhenium)
Be (Beryllium)	Cu (Copper)	Nb (Niobium)	I (Iodine)	Tb (Terbium)	Os (Osmium)
B (Boron)	Zn (Zinc)	Mo (Molybdenum)	Xe (Xenon)	Dy (Dyprosium)	Ir (Iridium)
Al (Aluminum)	Ga (Gallium)	Ru (Ruthenium)	Cs (Cesium)	Ho (Holmium)	Pt (Platinum)
Sc (Scandium)	Ge (Germanium)	Rh (Rhodium)	Ba (Barium)	Er (Erbium)	Au (Gold)
Ti (Titanium)	As (Arsenic)	Pd (Palladium)	La (Lanthanum)	Tm (Thulium)	Hg (Mercury)
V (Vanadium)	Se (Selenium)	Ag (Silver)	Ce (Cerium)	Yb (Ytterbium)	Tl (Thallium)
Cr (Chromium)	Kr (Krypton)	Cd (Cadmium)	Pr (Praseodymium)	Lu (Lutetium)	Pb (Lead)
Mn (Manganese)	Rb (Rubidium)	In (Indium)	Nd (Neodymium)	Hf (Hafnium)	Bi (Bismuth)
Fe (Iron)	Sr (Strontium)	Sn (Tin)	Sm (Samarium)	Ta (Tantalum)	Th (Thorium)
Co (Cobalt)	Y (Yttrium)	Sb (Antimony)	Eu (Europium)	W (Tungsten)	U (Uranium)

CHAPTER 5

THE DELIVERY SYSTEM RESULTS IN ADDITION AND LOSS OF TRACE ELEMENTS FROM A TPN SOLUTION

SPECIFIC AIM

The specific aim of this experimental section was to examine the extent of trace element alterations in delivered TPN solutions subjected to clinically relevant time and temperature variables.

INTRODUCTION

Home TPN has been established as a practical form of nutritional support for patients requiring long-term intravenous feeding. During home TPN the intravenous route often becomes the sole source for the acquisition of nutrients. It, therefore, becomes imperative that the solutions delivered to patients contain appropriate amounts of essential nutrients, including trace elements. TPN solutions are of extremely complex composition making them highly susceptible to compatibility and stability problems. Potential trace element interactions in TPN, therefore, become of therapeutic concern to clinicians.

Past reports of trace element deficiencies, such as Cr [1,2], Se [3], Cu [4], and Zn [5] during long term TPN were attributable to a lack of added trace elements to TPN solutions. During this early period of TPN use patients may have survived for prolonged periods without added trace elements because TPN component solutions were contaminated with essential trace elements. Deficiencies, however, developed over time as body stores were depleted in the face of inadequate intakes. Since the recognition of these deficiencies and the addition of trace elements to long term TPN solutions, there have been fewer reported cases of trace element deficiencies. It must be noted that there have been few studies examining the trace element status of long term home TPN patients. Due to the difficulties in measuring and interpreting trace element levels in biological fluids and tissues, it is likely that a number of marginal deficiencies of trace elements persist undetected. In clinical cases where trace element deficiencies have appeared, there are usually numerous factors that make it difficult to discern the cause of deficiency [6]. These include extraordinary fistula or diarrheal loses, decreased bioavailability in the body due to the presence of decreased binding proteins, and the presence of endogenous chemicals, medications, and other nutrients which may affect trace element levels in patients [7].

It has been suggested that the interaction of trace elements with other components in the TPN solution during storage, or with the container, administration tubing or filter, may also be responsible for inadequate delivery of trace elements and may result in deficiencies. Researchers have considered possible mechanisms of loss including precipitation and adsorption. Reactions within the nutrient solution may cause elements to precipitate or to be "complexed" in a form that is biologically unavailable to the host [8]. Most precipitates formed would be trapped by the in-line filter and would not be delivered. Certain chelating agents are present in some TPN component solutions. For example, EDTA is added to certain Ca-gluconate injections as a stabilizing agent [9]. These agents are heavy metal chelators, and may bind or precipitate trace elements such as Zn and Cr out of solution. There have been reports of an interaction between the amino acid cysteine and Cu in TPN solutions for infants in which Cu precipitates out of solution [10]. Whether or not other amino acids induce similar reactions with Cu or other trace elements have not been reported. Sodium selenite (selenious acid or selenium dioxide) is often used as a trace element additive for TPN solutions. Selenite can be reduced to elemental Se resulting in precipitation [11]. High concentrations of ascorbic acid have been reported to reduce selenite to elemental Se, which is nutritionally unavailable [12,13].

TPN solutions are in contact with a number of plastic components which may prevent the delivery of trace elements by adsorption to the plastic surfaces. Adsorption losses of trace elements from aqueous solutions on various containers have been studied. In general, adsorption losses for a number of trace elements (eg, Ba, Mn, Cd) have been found to increase with increases in pH [14]. Increases in pH and surface area increase the rate of Zn adsorption to a variety of materials [14,15]. Zinc adsorption was also shown to decrease with increasing storage time. However, this phenomenon could be due to the simultaneous leeching of Zn from the container materials, thereby, confounding the results. Most of the literature has concluded that Se is not likely to suffer serious adsorption losses [16]; however, Cr^{+3} losses from polyethylene containers were 25% of the original elemental concentration (1 μg/L) at pH 6.5 after storage 23°C for 15 days [17]. Few studies have examined the effect of in-line filtration on the retention of trace elements. Many in-line filters are made of various plastic polymers, including polyethylene. Studies have shown that a number of aqueous solutions of trace elements may be adsorbed to polyethylene: Al, Ba, Cr Co, Cu, Mn, Se, Sr and Zn [15]. Therefore, there exists the possibility that trace elements could be lost via adsorption to the filter components.

The results of trace element adsorption studies should be interpreted with the knowledge that few definitive investigations on exchange reactions between sample and container have been made. Studies with aqueous solutions are difficult to compare with TPN solutions since the compositions vary in complexity. For example, TPN solutions contain amino acids which are capable of binding certain trace elements. Zinc is known to chelate with amino acids [18,19]. There is no doubt that this occurrence must have some effect on the exchange of trace elements with the container. Additionally, very small concentrations of trace elements (eg, 1μg/L) have been studied in aqueous media, and only in those materials typically used to store trace element-containing samples. There is usually a greater concentration of trace elements present in TPN solutions than in the aqueous samples studied because of the need to deliver the daily recommended levels. No reports of the adsorption characteristics of polyvinyl chloride have appeared in the literature. Hence, the adsorption of trace elements to this commonly used medical grade plastic remains unknown.

There is no question that TPN component solutions as purchased from the manufacturer are contaminated with a variety of trace elements, both essential and nonessential. This has been repeatedly demonstrated in the literature (and was confirmed in Chapter 3 of the thesis). We have previously shown that compounding TPN solutions also adds Zn as a trace element contaminant. Whether or not the delivery system—the TPN bag, adminstration tubing, and the in-line filter–contribute any trace element contaminants has not been specifically addressed in past studies.

From the time of compounding and throughout delivery, the TPN solution is in contact with plastic components, namely the polyvinyl chloride TPN bag and administration tubing, and the polyethylene (in this particular study) in-line filter. During the manufacturing of plastic, trace element-containing chemicals are added to obtain desired characteristics, as described previously (Chapter 3 of the thesis). As a result, some polyvinyl chloride containers have been shown to contain low levels of trace elements such as Zn [20]. This observation was confirmed in the compounding experiment described in Chapter 4 of the thesis. Polyvinyl chloride and polyethylene have been previously shown to be contaminated with trace elements such as Zn and Sb, and Zn and Si, respectively [21]. Elastomeric sealants and closures are used in TPN delivery systems to access the contents of the TPN bag. The wide variety of trace element-containing chemicals added to produce the desired shape or consistency of sealant have previously been described in Chapter 3 of the thesis. Trace elements may be leeched from the plastic and elastomers during storage or delivery through the adminstration tubing, and ultimately reach the patient. The degree to which this problem occurs likely depends on how long the plastic or elastomer is in contact with a solution. Since the tubing of the compounding equipment is also made of polyvinyl chloride, it is possible that the passage of the separate component solutions through the plastic lines may leech trace elements along the way. This phenomenon has yet to be formally tested.

Metal molds and extrusion equipment are used to manufacture plastic and rubber materials. The metal in contact with the plastic may be of many different alloys. Therefore, during manufacturing elements may be leeched from the metal into the plastic and rubber components. Upon exposure of TPN solutions to the plastic and rubber, these elements may then be leeched into the solution.

No studies have attempted to specifically examine in-line filters for the presence of trace elements which may leech or be eluted upon contact with a parenteral solution. In one of the few studies which considered trace element contamination by in-line filters, only the elements directly under investigation (eg, elements added as part of single or multielemental intravenous additives) were considered as potential contaminants of the in-line filters [22]. No attempts were made to search for other contaminants.

The toxicity of intravenously infused trace elements remains largely unknown. Therefore, the long-term effects of infusing contaminating and essential trace elements in TPN solutions and how this affects a patient's trace element status remains to be determined.

A number of home TPN patients are provided with premixed, patient-specific TPN solutions supplied up to 30 days in advance of use. Patients only have to add one or two substances (eg, multivitamins) to the premixed solution prior to delivery. Such situations have increased the interest in the stability of trace elements in TPN solutions over time, specifically, whether or not the component additives and storage container and conditions alter the trace element content of the solution received by the patient at the time of delivery. During containment, solutions may change in trace element composition due to adsorption or leeching of trace elements, as described above. It is likely that storage duration and temperature also have effects on the trace element composition of TPN solutions. Since neither completely pure nor completely inert materials are known, both leeching gains and adsorption losses of trace elements should be considered during the storage of TPN for patients [23]. There has been little data generated regarding trace element compatibility and stability beyond 48 hours. This is inadequate for long-term home TPN patients who may keep formulas for up to one month.

This study seeks to provide information regarding trace element stability over a duration which most accurately represents the experience of home TPN patients (ie, 36 hours to 30 days after compounding). The study was designed to provide information regarding the preservation of intended trace element concentrations in solutions exposed to storage conditions typically imposed by home TPN patients, and to discern potential leeching and adsorption phenomena which may alter trace element concentrations in TPN solutions. Specifically, this study sought to determine (1) the effect of time, ie, early (36 hours) or late (30 days) delivery following compounding and (2) storage temperature, ie, 4°C or 20°C on trace element concentrations in TPN solutions.

MATERIALS AND METHODS

Supplies

Sixteen 1000 mL pthalate-free TPN bags (Exacta-Med Container, Baxa, Englewood, CO, 80112; Lot 4H03507) of experimental TPN solution (Table 5-1) were compounded (Baxa MicroMacro 23 Compounder with IEEE 488 Interface, Englewood, CO, 80112) at University of Alberta Hospitals Pharmacy using the TPN components purchased as Lot 1 (See Table 3-1, Chapter 3). Peristaltic infusion pumps (Flo-Guard 6300 Dual Channel Volumetric Infusion Pump, Baxter, Deefield IL) and disposable solution administration sets with 0.22μ downstream 72 hour depth filters (Basic Solution Set 2C5493S, Baxter, Deerfield, IL) were employed for delivery of the TPN solutions. Polypropylene collection bottles were used to collect the delivered TPN solution (Narrow-mouthed bottle, Nalge Company, Rochester, NY).

Methods

New collection bottles were rinsed with Nanopure trace element-free water (as described in Chapter 2), soaked overnight in Extran 300 concentrated cleaning solution (BDH Chemicals, B80002), and then rinsed again with Nanopure trace element free water. Twenty-five millilitres of pure trace element free nitric acid (Baker Company, Instra-analyzed for trace metal analysis, 9598-00) was added to each bottle and swirled well. Each bottle was rinsed well with Nanopure trace element free water and left to dry in a HEPA-filtered hood.

Stability of Trace Elements With Freezing

Prior to commencing the delivery of the experimental TPN solutions the trace element concentrations in the TPN solution were measured before and after freezing. This was performed to validate the stability of trace elements for purposes of sample storage at reduced temperatures. A 500 mL TPN bag of the experimental TPN solution was compounded and stored refrigerated (4°C) overnight. Approximately sixteen hours later the bag was warmed to room temperature under warm running water, and 2.78 mL adult MVI solution was added. After the solution was thoroughly mixed, eight 30 mL samples were taken from the medication port of the bag with 60 mL syringes and needles (Becton-Dickenson, Rutherford, NJ, 07070). The needles were removed before ejecting the solution into high density polypropylene vials (Sarstedt, St. Laurent, PQ). Four samples were immediately analyzed for trace elements using the quantitative setting of the ICP-MS. The other four samples were frozen for approximately one week at -70°C. Prior to analysis, samples were thawed and well homogenized.

Stability of Trace Elements During Delivery

A pilot experiment was then performed to determine whether or not any change in trace element concentration occurred in the TPN bag or delivered solution during the ten hour delivery period. Any new contaminants potentially leeching or eluting from the infusion tubing or the TPN bag into the delivered solution would also be identified at this time. One 1000 mL bag of TPN solution was compounded at the University of Alberta Hospitals Pharmacy according to the experimental formula (Table 5-1) and refrigerated overnight. Unknown lots of TPN components were used for the purposes of the experiment. Approximately sixteen hours later the TPN bag was warmed to room temperature under warm running water and 5.56 mL adult MVI solution was added. The TPN bag was suspended from an IV pole and spiked with an adminstration set according to the manufacturer's instructions. The end of the administration tubing was placed inside a HEPA-filtered hood where it was suspended over a collection bottle by a three-pronged clamp. A Flo-Guard infusion pump was set to deliver at a rate of 60 mL/hr for a period of ten hours. Two samples were taken at each time period from the TPN bag (0, 0-1, 1-2, 2-4, 4-6, 6-8, 8-10 hours) and the collection bottle (0-1, 1-2, 2-4, 4-6, 6-8, 8-10 hours).

TPN Delivery and Sample Collection

Immediately after compounding the 16 TPN bags of experimental solution, eight were placed in a storage cupboard (20°C) and eight were refrigerated (4°C). Approximately 36 hours later, four bags were removed from the storage cupboard and four from the refrigerator for delivery in the "early" period. The remaining eight bags were kept stored at their respective temperatures for approximately 30 days. In 30 days four bags were removed from the cupboard (20°C) and four from the refrigerator (4°C) for delivery in the "late" period.

Refrigerated bags were warmed to room temperature by running under warm water. Adult MVI (5.56 mL) was added to the medication port of each bag with a syringe and needle (Becton-Dickinson, Rutherford, NJ, 07070) and mixed well. The TPN bags were suspended from IV poles and the adminstration tubing was prepared according to the manufacturer's instructions. The end of each set of administration tubing was placed inside a HEPA-filtered hood where it was suspended over a collection bottle by a three-pronged clamp. The Flo-Guard pumps were set to deliver at a rate of 60 mL/hr for a period of ten hours. The delivered solution was collected at 0-1, 1-2, 4-6 and 8-10 hours (Figure 5-1). Thirty millilitre samples of delivered solution were taken from the collection bottles of each bag at each time period and poured into high density polypropylene vials. Non-powdered gloves were used as a precaution against extraneous contamination throughout the experimental delivery, including set-up and sampling. All samples were immediately frozen at -70°C for two weeks prior to analysis.

Trace Element Analysis

Samples were analyzed for trace elements using ICP-MS (Perkin Elmer Sciex Elan 5000A ICP-MS System, Perkin Elmer Inc. Toronto, ON). For the pilot experiment, samples were analyzed in two parts. First, a "Spectrum-directed" analysis for 66 trace elements (Table 5-2) was performed using the Total Quant Mode (scanning from atomic mass 3[Li] to 92[U]; excluded were major elements) to screen for trace element contaminants. Based upon the results of the "Screen" another set of samples underwent an "Element-directed" quantitative analysis employing the Quantitative Mode of the ICP-MS. For the delivery experiment, four delivered samples collected per TPN bag (corresponding to the collections made at the four time periods during delivery) were analyzed using the "Quant" setting for any trace element appearing at a level $>1\mu g/L$. Iodine (I), although it was part of the MTE-6 trace element additive, was not analyzed during this study because the ICP-MS instrument available for analysis was not configured to measure anionic species.

Data Analysis

Results are reported as means ± SEM of four TPN bags per experimental time/temperature variation (as represented in Figure 5-1). T-tests were used to identify significant differences in trace element concentrations at the various time/temperature combinations and with calculated amounts of trace elements contained in Lot 1 solution components. Statistical analyses were performed with Sigma Stat statistical software package (Sigma Stat version 1.00, Jandel Scientific, San Rafel, CA, 94912). A probability value of $p<0.05$ was considered to be significant.

RESULTS

Stability of Trace Elements With Freezing

Results of trace element analysis indicated that there was no change in trace element concentration with freezing and storage at -70°C (Data not shown). Therefore, this was confirmed as a suitable method of sample storage

Stability of Trace Elements During Delivery

Cobalt (Co) appeared as the only new trace element contaminant (ie, in addition to those 11 already present in Lot 1 components: Zn, Cu, Mn, Cr, Se, B, Al, Ba, V, Ti, Sr). During the ten hour delivery period there were no significant changes in trace element concentration in the TPN solution from the bag or collected from the delivered solution. This suggested that trace elements were stable in the TPN solution over the 10 hour delivery period. (The mean trace element concentration of the four delivered collection periods, 0-1, 1-2, 4-6 and 8-10 hours, was therefore used to represent the mean trace element concentration delivered from each TPN bag.) There was no significant difference in the concentration of trace elements from the TPN bag compared to the delivered solution after storage at 4°C, and sampling approximately sixteen hours after compounding. Therefore, any reaction(s) responsible for the alteration of trace element concentrations in the TPN bag or delivery system would have had to occur after this time.

Delivery Experiment: Time/Temperature Factors

Prior to determining whether or not trace elements were added or lost during delivery, we needed to calculate the exact amount of trace elements (the sum of the intentional additives and contaminants) in the TPN solution. The concentration of individual trace elements in the TPN solution were thus calculated by totalling the amounts contained within each of the nine components from Lot 1 used to make up the TPN solution. This "calculated" amount represents the level of trace elements present as the sum of intentional additives (Zn, Cu, Mn, Cr, Se) and as contaminants (B, Al, Ba, V, Ti, Sr, Co) before any manipulation of the solution. The loss or addition of trace elements is thus determined relative to this "calculated" value.

Zinc, Cu, Mn, Se, and Cr: essential trace elements subject to loss from the TPN solution during delivery

The measured Zn concentration delivered from a TPN solution stored at 4°C for 36 hours (early delivery) and 30 days (late delivery) was significantly less than that calculated ($p<0.0008$, $p<0.0001$, respectively). In addition, Zn concentration after late delivery was significantly less than after early delivery ($p<0.001$). The measured Zn concentration delivered from a TPN solution stored at 20°C for 36 hours (early delivery) and 30 days (late delivery) was significantly less than the calculated ($p<0.0001$, respectively) (Figure 5-2).

The measured Cu concentration delivered from a TPN solution stored at 4°C for 30 days (late delivery) was significantly less than that in the solution stored for 36 hours (early delivery) ($p<0.004$) and the calculated ($p<0.0001$). The measured Cu concentration delivered from a TPN solution stored at 20°C for 36 hours (early delivery) and 30 days (late delivery) was significantly less than the calculated ($p<0.003$, $p<0.0006$, respectively). (Figure 5-3).

The measured Mn concentration delivered from a TPN solution stored at 4°C for 30 days (late delivery) was significantly less than that in the solution stored for 36 hours (early delivery) ($p<0.01$) and the calculated ($p<0.0001$). The measured Mn concentration delivered from a TPN solution stored at 20°C for 36 hours (early delivery) and 30 days (late delivery) was significantly less than the calculated ($p<0.0005$, $p<0.0001$, respectively). (Figure 5-4).

The measured Se concentration delivered from a TPN solution stored at 4°C for 36 hours (early delivery) and 30 days (late delivery) was significantly less than the calculated ($p<0.03$, $p<0.0001$, respectively). The measured Se concentration delivered from a TPN solution stored at 20°C for 36 hours (early delivery) and 30 days (late delivery) was significantly less than the calculated ($p<0.04$, $p<0.0001$, respectively). In addition, Se concentration after late delivery was significantly less than after early delivery ($p<0.01$). (Figure 5-5).

Significant changes in Cr concentration compared to calculated concentrations from Lot 1 and delivery time and temperature variables, were not able to be quantified using the standard equipment on the ICP-MS instrument available for analysis due to interfering organic compounds in the dextrose and Travasol components. Results indicated a trend toward a decreased Cr concentration compared to values calculated from Lot 1 for each time/temperature combination, and decreased concentration from early to late delivery at both storage temperatures (Data not shown).

Boron and Al: trace element contaminants subject to loss from the TPN solution during delivery

The measured B concentration delivered from a TPN solution stored at 4°C for 36 hours (early delivery) and 30 days (late delivery) was significantly less than the calculated ($p<0.0001$, respectively). Additionally, B concentration after late delivery was significantly less than after early delivery ($p<0.0005$). The measured B concentration delivered from a TPN solution stored at 20°C for 36 hours (early delivery) and 30 days (late delivery) was significantly less than the calculated ($p<0.0001$, respectively). Boron concentration after late delivery was significantly less than after early delivery ($p<0.0001$). (Figure 5-6).

The measured Al concentration delivered from a TPN solution stored at 4°C for 36 hours (early delivery) and 30 days (late delivery) was significantly less than the calculated ($p<0.0001$, respectively). In addition, Al concentration after late delivery was significantly less than after early delivery ($p<0.002$). The measured Al concentration delivered from a TPN solution stored at 20°C for 36 hours (early delivery) and 30 days (late delivery) was significantly less than the calculated ($p<0.0001$, respectively). Aluminum concentration after late delivery was significantly less than after early delivery ($p<0.002$). (Figure 5-7).

Barium, V and Ti: trace element contaminants subject to addition to the TPN solution during delivery

The measured Ba concentration delivered from a TPN solution stored at 4°C for 36 hours (early delivery) and 30 days (late delivery) was significantly greater than the calculated ($p<0.0001$, respectively). The measured Ba concentration delivered from a TPN solution stored at 20°C for 36 hours (early delivery) and 30 days (late delivery) was significantly greater than the calculated ($p<0.0001$, respectively). Additionally, Ba concentration after late delivery was significantly less than after early delivery ($p<0.02$). (Figure 5-8).

The measured V concentration delivered from a TPN solution stored at 4°C for 36 hours (early delivery) and 30 days (late delivery) was significantly greater than the calculated ($p<0.0009$, $p<0.01$, respectively). In addition, V concentration after late delivery was significantly less than after early delivery ($p<0.01$). The measured V concentration delivered from a TPN solution stored at 20°C for 36 hours (early delivery) and 30 days (late delivery) was significantly greater than the calculated ($p<0.005$, $p<0.009$, respectively). (Figure 5-9).

The measured Ti concentration delivered from a TPN solution stored at 4°C for 36 hours (early delivery) and 30 days (late delivery) was significantly greater than the calculated ($p<0.0001$, respectively). The measured Ti concentration delivered from a TPN solution stored at 20°C for 36 hours (early delivery) and 30 days (late delivery) was significantly greater than the calculated ($p<0.0001$, respectively). Titanium concentration after late delivery was significantly greater than after early delivery ($p<0.006$). (Figure 5-10).

Strontium and Co: trace element contaminants which are not subject to alteration in the TPN solution during delivery

The measured Sr concentration delivered from a TPN solution stored at 4°C for 36 hours (early delivery) and 30 days (late delivery) was significantly greater than the calculated ($p<0.0001$, respectively). The measured Sr concentration delivered from a TPN solution stored at 20°C for 36 hours (early delivery) and 30 days (late delivery) was significantly greater than the calculated ($p<0.0001$, respectively). (Figure 5-11).

The measured Co concentration delivered from a TPN solution stored at 4°C for 36 hours (early delivery) and 30 days (late delivery) was significantly greater than the calculated (ie, calculated = 0 μg/L) ($p<0.0001$, respectively). The measured Co concentration delivered from a TPN solution stored at 20°C for 36 hours (early delivery) and 30 days (late delivery) was significantly greater than the calculated ($p<0.0001$, respectively). (Figure 5-12).

DISCUSSION

Zinc, Cu, Mn, Se and Cr: essential trace elements subject to loss from the TPN solution during delivery

At each delivery time/temperature combination examined in this study, Zn and Se concentrations were shown to be significantly lower than the amount calculated (intentionally added and contaminant) to be present in the TPN solution (Figures 5-2 and 5-5). Chromium could not be accurately quantified with the standard equipment of the ICP-MS instrument available because of problems with interfering organic components in the TPN solution. However, at each delivery time/temperature combination examined in this experiment, Cr concentration also appeared to be lower than the calculated (No figure). (Lot 1 Cr concentration was calculated using values from graphite furnace atomic absorption spectrometry analysis for dextrose and Travasol quantified at Quebec Centre of Toxicology. Both solutions showed reported values of <2.0 μg/L). The concentrations of Cu and Mn following early and late delivery after storage at 20°C, and following late delivery after storage at 4°C, were significantly less than the calculated (Figures 5-3 and 5-4). These results suggest a loss of the aforementioned elements from some point in the delivery system. The observed losses could have resulted from precipitation of the trace elements from the TPN solution, or adsorption reactions with the compounding tubing, TPN bag, administration tubing, or in-line filter. In this study, 556 mg/L ascorbic acid was present in the TPN solution as part of the adult MVI additive along with 560 μg Cu. Both Cu and vitamin C have been shown to reduce selenious acid to elemental Se which may precipitate from solution in addition to becoming nutritionally unavailable [24]. This may partially explain the lower Se concentration of the delivered solutions compared to the calculated. Aqueous solutions of Zn, Cu, Mn, and Cr have been shown to be adsorbed to plastic materials such as polyethylene, which was the material of the in-line filter used in this study [15]. It is unlikely that Se was bound to the polyvinyl chloride plastic TPN bag or tubing of the administration set, or the polyethylene of the in-line filter since little research has supported Se adsorption to plastic materials. Adsorption of Zn, Cu, Mn and Cr to polyvinyl chloride has not been studied.

The concentrations of Cu and Mn following early delivery and storage at 4°C, were the same as the calculated. This suggests that there was likely no adsorptive losses of these trace elements during compounding. Additionally, it appeared that no detectible reactions affecting Cu and Mn were occurring in the bag or delivery system during this time period. Copper and Mn, therefore, appear stable in TPN solutions delivered 36 hours after compounding and storage at refrigerated temperatures (4°C). It would not be expected that Zn would be adsorbed to the polyvinyl chloride tubing of the compounder, since findings in Chapter 3 of the thesis revealed that compounding and the TPN bag appear to add small amounts of Zn. It is possible that Zn was added to the solution by the compounding equipment as well as the TPN bag, but by the time the solution was delivered 36 hours after the compounding procedure, various reactions could have reduced Zn concentrations. It is unlikely that the initial concentration of Cr, was reduced by adsorption to compounding equipment, due to the short contact time of the component solutions with the compounding materials.

When TPN solutions were stored at 4°C, significant falls in Zn, Cu and Mn concentrations were observed after late delivery compared to early delivery. In contrast, no significant difference was observed in Zn, Cu, and Mn concentrations when early and late delivery were compared following storage at 20°C. This suggests that at 20°C, the reaction(s) responsible for the reduction of Zn, Cu, and Mn appear to occur some time before or during delivery in the early period (ie, 36 hours after compounding), with no further detectible reduction in the elements with prolonged storage. This can be inferred from the apparent, but not statistically significant reduction of Zn Cu and Mn concentration ($p=0.056$, $p=0.052$, $p=0.06$, respectively) after early delivery at 20°C compared to early delivery at 4°C. A larger sample size would likely provide statistical significance. Storage of TPN solutions at 4°C appears to delay the reduction of Zn, Cu, and Mn concentrations in the early delivery period.

When TPN solutions were stored at 20°C, a significant reduction in Se concentration was observed after late delivery compared to early delivery. Changes in the TPN solution over time (eg, pH changes, precipitation, changes due to degradation of solution components) may have facilitated such a reduction. After storage of TPN solutions at 4°C, there was no significant difference in Se concentration when comparing early and late delivery concentrations. This suggests that the reaction(s) responsible for the reduction of Se at 4°C appear to occur some time before or during delivery in the early period (36 hours after compounding), with no further detectible reduction in Se with prolonged storage. This can be inferred from the apparent reduction of Se concentration after early delivery at 4°C compared to early delivery at 20°C. Storage of TPN solutions at reduced temperatures, may render Se less soluble and induce its precipitation out of solution. These precipitates would be trapped by the in-line filter. Storage of TPN solutions at 20°C appears to delay a reduction in Se concentration in the early delivery period.

Chromium concentration appeared to decrease after late delivery compared to early delivery following storage at both 4°C and 20°C (Data not shown). This suggests that prolonged storage (ie, up to one month) may reduce the delivered level of Cr. This observation may have ensued because of reactions occurring within the solution (eg, precipitation) or with the delivery components (eg, binding to plastic components).

Clinical Relevance. Zinc, Cu, Mn, Se, and Cr are essential nutrients for the human organism. When analyzing the clinical relevance of the decreased delivered Zn, Cu, Mn, Se and Cr concentrations after prolonged storage, it becomes clear that there is little cause for concern. Current daily parenteral Zn, Cu, Mn, and Cr recommendations for the stable adult are 2500 to 4000 μg, 500 to 1500 μg, 150 to 800 μg, and 10 to 15 μg, respectively [25]. There is no official parenteral recommendation for Se by the AMA or any other collective authority. Recommendations for parenteral Se in home TPN patients vary from 40 to 120 μg/day [8]. From Table 5-3 it is evident that based upon delivery of two litres of the experimental TPN solution per day, the concentration of delivered Zn at each of the time/temperature combinations exceeds the recommended parenteral requirements, and the concentration of delivered Cu, Mn, and Se remains within the range of parenteral requirements. Given the problems with the delivered values obtained for Cr, it is not possible to determine whether or not Cr requirements were met, exceeded, or deficient in early or late delivery periods, and after storage at either temperatures. In general, even after a month of storage at either temperature, a patient will likely receive recommended levels of Zn, Cu, Mn and Se. However, this depends somewhat on the amount of trace element contamination present in the TPN component solutions. As demonstrated in Chapter 3 of the thesis, there is significant inter-lot variation in the trace element content of the component solutions. This would affect the amount ultimately delivered to a patient depending on the contamination present in a particular lot used to prepare a prescribed TPN formula. The contamination of solution components by Zn, Cu, Mn, and Se may aid in cushioning any reductions which may occur as a consequence of the delivery system.

Boron and Al: trace element contaminants subject to loss from the TPN solution during delivery

Boron and Al were not present as intended constituents of the MTE-6 trace element additive. They were contaminants of the Lot 1 components used to produce the experimental TPN solution. At each delivery time/temperature combination studied, B and Al concentrations were significantly lower than the calculated (Figures 5-6 and 5-7). It, thus, appears that the level of contamination originally present in the TPN component solutions was significantly decreased after delivery: only 12% of the original B, and 43% of the original Al concentration was delivered. Boron and Al concentrations decreased after late delivery compared to early delivery following storage at both 4°C and 20°C. This implies that prolonged storage (ie, after 30 days) can reduce the delivered level of these trace element contaminants. These observations could be the result of reactions occurring within the solution (eg, precipitation) or reactions with the delivery components (eg, adsorption to plastic). It seems unlikely that the compounding process would reduce initial B and Al concentrations to such a low level compared to the calculated values from Lot 1 components. In Chapter 4 of the thesis, it was shown that there was no reduction of B or Al when passed through the compounding system as contaminants of sterile water. The B concentration calculated from Lot 1 components is quite high compared to the final delivered amounts. It is possible that a sample from the analyzed component solutions was contaminated with B. Aluminum has been shown to adsorb to plastic (polyethylene) when in aqueous solutions [15]. It is possible that some of the Al was adsorbed to the in-line filter. No studies have examined the adsorption of trace elements such as B and Al to polyvinyl chloride materials. Thus, the interaction of these elements with the plastic components of the TPN delivery system remain largely unknown.

Clinical Relevance. Since B and Al are considered to be contaminants, it would be appropriate to describe the above "reduction" reactions as being beneficial to the patient. Since neither element is considered essential for humans, there are no parenteral intravenous requirements. However, when compared to amounts ingested orally and absorbed (Table 5-3) it is clear that the B delivered after each experimental time/temperature combination is substantially less. This may actually have implications for B balance in the body. For example, an inadequate intake of B has been suggested as a an important factor in the etiology of osteoporosis [26]. Boron appears to have beneficial effects on Ca metabolism and in the prevention of bone loss [27]. Considering the number of skeletal abnormalities reported in patients receiving long term TPN, these findings may hold some relevance. More work needs to be done in this area to confirm the relationship of this trace element in bone metabolism.

There have been numerous accounts of the deleterious effects of Al on patients, including osteomalacia and dementia, resulting from Al contamination of TPN solutions [28]. The amount of Al delivered via intravenous contamination in each time/temperature delivery period appears to meet or exceed the amounts ingested and absorbed from the oral diet (Table 5-3). This comparison is based upon a reported intake of 20,000μg Al. However, the oral diet can vary greatly in Al content, from 1000 to 30,000 μg/day [29]. Based upon this intake range, TPN patients receiving the delivered amounts reported in this study could easily exceed the oral daily intake. Excessive Al accumulation in the body has been reported with both oral and parenteral exposure. Aluminum toxicity in adults and infants has been documented as a result of direct parenteral exposure where the protective barrier of the gastrointestinal tract has been bypassed. Tissue accumulation, reduced bone formation, and osteomalacia have been reported in adult patients with normal or near normal renal function receiving long term TPN [30]. Despite the substantial reduction of Al contamination effected by the delivery system, the amounts remaining for delivery to patients present a cause for concern.

Barium, V, and Ti: trace element contaminants subject to addition to the TPN solution during delivery

Barium, V, and Ti were not present in the MTE-6 trace element solution as intentionally added elements. Rather, they were present in the Lot 1 solution components as contaminants. The concentrations of Ba, V, and Ti appearing in the delivered TPN solution at each time/temperature period were significantly greater than the calculated (Figures 5-8, 5-9, and 5-10). This suggests that additional Ba, V, and Ti contamination was derived from the delivery system, TPN bag, or alternatively, the compounding equipment. Approximately 16 μg/L Ba, 2.75 μg V, and 52 μg Ti were added to that already present in the Lot 1 solution components. It is possible that these elements were leeched from the compounder as the TPN component solutions passed through the plastic lines. However, this seems unlikely due to the minimal contact time of the solution components with the compounding device (ie, 15 seconds per solution). The additional Ba, V, and Ti could have leeched from the TPN bag, administration set tubing, or filter components. The experiment performed in Chapter 4 of the thesis did not detect any Ba, V, or Ti in the TPN bag compounded with sterile water. However, this does not rule out the possibility that leeching of these elements from the TPN bag could have occurred with exposure of the bag to the entire mixed solution, especially after prolonged storage.

During the manufacturing of polyvinyl chloride plastic TPN bags and administration tubing, plastic pellets are extruded at high temperatures into sheets or tubing. It is very possible that the metallic extruding equipment used in the manufacturing process contained Ti metal. Titanium metal is primarily used in the production of high-strength, corrosion resistant alloys. This would be an important characteristic for equipment used to manufacture medical devices. In addition, Ba and V are used in the manufacture of alloys and special steels, respectively. During the high temperature extrusion, some Ti, Ba, or V metal may infiltrate the plastic, only to be leeched out with exposure to the TPN solution. Additionally, these elements may remain on the plastic as residues to be eluted upon exposure to the TPN solution. With prolonged exposure (eg, TPN solution stored in a polyvinyl chloride bag) more of these trace elements may be leeched into the solution. Barium and V are also added during the manufacture of various plastics and rubber to obtain certain characteristics. Upon contact of the TPN solution with plastic and rubber, especially after prolonged storage, Ba and V could have leeched into the solution. Titanium-dioxide is extensively used in the manufacture of plastics where it imparts a white pigment to the product [31]. The in-line filter of the administration set is made of polyethylene. The brilliant white of the filter is characteristic of plastics manufactured with Ti dioxide. It is possible that Ti could have been leeched from the filter as the TPN solution advanced through it. The TPN solution was propelled through the tubing with the assistance of a peristaltic infusion pump. It is possible that the massaging motion of the pump on the plastic tubing assisted in releasing Ti from the plastic. Handling and movement of plastic devices has been shown to lead to a greater release of particulates [32]. However, these last two observations do not explain the increased Ti observed after prolonged storage at 20°C (ie, significant increase during late delivery compared to early delivery), unless various chemical changes in the solution favoured increased leeching by the filter or tubing.

There were no significant differences between the Ba concentrations of solutions delivered early or late after storage at 4°C. However, after storage at° 20 C, the Ba concentration of the solutions delivered in the late period were significantly less than those delivered in the early period (Figure 5-8). There was no significant difference between the V concentrations of solutions delivered early or late after storage at 20°C. However, there was a significant reduction in V concentration after late delivery compared to early delivery at 4°C

(Figure 5-9). Various reactions, such as precipitation out of solution after prolonged storage at elevated (in the case of Ba) or reduced (in the case of V) temperatures, and binding to plastic components of the delivery system or TPN bag, could have been responsible for these observations. There have been no studies which have examined the adsorption of Ba or V to polyvinyl chloride plastics.

There was no significant difference between the Ti concentrations of solutions delivered early or late after storage at 4°C. However, after storage at 20°C, the Ti concentration of the solutions delivered in the late period were significantly greater than those delivered in the early period (Figure 5-10). It appeared that more Ti was leeched out of the delivery system components or TPN bag after prolonged storage at elevated temperature (20°C). The most plausible source for the increased Ti would appear to be leeching from the TPN bag. Storage at 4°C may have inhibited the leeching reaction.

Clinical Relevance. Since Ba, V, and Ti are considered to be contaminants of TPN solutions, and are not essential nutrients, there are no recommended levels for intravenous infusion. The levels of Ba, V, and Ti delivered in two litres of solution at each time/temperature period in this experiment exceed the amounts absorbed from the oral diet (Table 5-3). Intravenously delivered Ba was 2 to 3 times greater than the amount absorbed from the oral diet, while V was 88 to 240 times, and Ti was 6 to 60 times more abundant. There is always an increased opportunity for toxicity to develop when the protective barrier of the gastrointestinal tract is bypassed. Barium is considered to have a low order of toxicity by the oral route. Intravenous toxicity levels are not known. The effects of Ba in biological systems depends upon the solubility of the salt in question since soluble salts are extremely toxic to living cells. Most Ba salts are insoluble in water. Ingestion of Ba doses as low as 0.2 to 0.5g (3 to 7 mg/kg body weight) have been found to lead to toxic effects in humans [33]. Clinical features of oral Ba poisoning include gastroenteritis, loss of deep reflexes with onset of muscular paralysis, and progressive muscular paralysis. Results of limited epidemiological studies relating exposure to low levels of Ba with cardiovascular disease and mortality were inconsistent and inconclusive [33]. The metabolism of Ba is similar to that of Ca. Most of the absorbed Ba is deposited in bone. Since numerous patients treated with prolonged TPN develop metabolic bone diseases, there is the possibility that Ba may be responsible for such pathology. Long-term TPN patients comprise a subpopulation in which there appears to be a higher than normal exposure to Ba. The effects of long-term intravenous administration of Ba are unknown. Thus, the potential for adverse health effects should be considered.

Due to poor absorption from the gastrointestinal tract, V is not very toxic to humans when ingested, but when introduced intravenously in a soluble form, the lethal dose for a 70kg human is only 30 mg V-pentoxide (0.42 mg V_2O_5/kg body weight) [34]. The toxicity of V varies with the nature of the compound. Chronic exposure to high levels of V results in cardiovascular effects such as arrhythmia, bradycardia and coronary spasm; hepatic effects such as enlarged liver and decrease in functional tests with bilirubinemia; reduced blood levels of vitamin C; and tendency toward anemia. Elevated blood, and especially urinary levels, provide documentation of V exposure. Unfortunately, in this study the exact source of contamination of V could not be delineated.

Little information exists with respect to the absorption, metabolism, and toxicology of Ti for humans. Reference levels in biological fluids have not been determined [35]. It has been demonstrated that Ti passes the blood-brain-barrier and that high concentrations appear to collect in pulmonary tissues regardless of inhalation exposure [31]. It has been questioned whether evidence of a specific oral Ti toxicity has ever been found [36], and intravenous toxicity has never been investigated. There have not been any previous reports of the presence of Ti in TPN solutions. Titanium metal and Ti metal containing alloys have been widely used in the production of medical prosthetic devices without any adverse local effects on tissues from the release of Ti [31]. This suggests that Ti is a biologically compatible element. Available data do not suggest that Ti or Ti compounds induce any mutagenic, carcinogenic or adverse immunologic effects [31]. At present, however, it is not possible to establish any dose-effect or dose-response relationships for any effect associated with exposure to Ti or its compounds. No quantitative assessment of human health risk can be made.

It appears imperative to pursue these enigmatic elements to ensure the health and safety of the long-term parenteral patient. No accounts of Ba, V or Ti contribution by the delivery system have been previously reported in the literature.

Strontium and Co: trace element contaminants which are not subject to alteration in the TPN solution during delivery

Strontium and Co were not present in the MTE-6 trace element solution. Strontium existed as a contaminant of the Lot 1 solution components and Co was exclusively a contaminant of the delivery system, TPN bag or compounding components. The concentrations of Sr and Co in the delivered TPN solution at each time/temperature period was significantly greater than the calculated (Figures 5-11 and 5-12). (The calculated amount of Co from Lot 1 was 0 μg/L.) This suggested that the additional Sr contamination came from delivery system, TPN bag, or the compounding equipment. Approximately 8μg of Sr was added to that present in the Lot 1 solution components. The results of the compounding experiment described in Chapter 4 of the thesis, indicated that no trace elements were leeched from TPN bags compounded with sterile water, apart from Zn. However, the addition of the formulated TPN solution to the bag may have induced the leeching of other trace elements, such as Sr and Co. There exists the possibility that Sr and Co were leeched from the plastic lines of the compounder. However, this seems unlikely due to the minimal contact time of the solution components with the compounder during compounding. There was no significant difference in the Sr and Co concentrations of solutions delivered early or late after storage at either 4°C or 20°C. Therefore, no changes in Sr and Co concentration occurred as a result of the time/temperature variables. The sources responsible for the increased concentrations of these elements were likely leeching from the TPN bag, administration tubing, or in-line filter upon contact with the TPN solution.

Cobalt is used in the manufacture of metal alloys where strength and resistance to oxidation are necessary [37]. It is possible that the extrusion equipment used in the manufacturing of medical polyvinyl chloride plastics contains Co. Like Ti, Co may penetrate the plastic during manufacturing, and be leeched out when in contact with the TPN solution. Strontium is used in the manufacturing of plastics [38] and Sr may likewise leech from the plastic components of the TPN bag, compounding and administration tubing or filter components upon contact with the TPN solution.

Clinical Relevance. Cobalt is an essential element for proper functioning of vitamin B_{12} in the human. Clinically, Co requirements are met in those receiving home TPN by providing sufficient amounts of intact vitamin B_{12}. Therefore, Co supplementation during TPN is unnecessary. When compared to the amount of Co ingested and absorbed from the oral diet, the amount of contaminating Co in two litres of delivered TPN solution was within the normal range of intake (Table 5-3). The intravenous toxicity of this element is not known. However, there is likely no risk of toxicity associated with the low level of infusion observed in this study.

There is no evidence to indicate that Sr is essential for humans. It appears as a contaminant of the oral diet. Compared to the reported oral intake, the intravenously delivered amount in two litres of solution is substantially less (Table 5-3). Limited information is available regarding the oral toxicity of stable Sr. The intravenous level for toxicity is unknown. The element is generally regarded as possessing a wide margin of safety between the dietary levels ingested as part of the food and water supply, and the levels that induce toxic effects. Strontium is poorly retained by humans and is efficiently eliminated by the kidneys [36]. It is, therefore, unlikely that the intravenously infused concentrations of Sr observed in this study are capable of producing any ill effects, even in the long term.

Inferences regarding the dietary intakes of trace elements and their comparisons with intravenous delivered amounts must be interpreted with care. The dietary intake of trace elements can vary widely depending on the proportions of various foods in the diet and the trace element content of foods grown in different geographical locations with varying soil distributions of trace elements. Due to the variability of trace element intake, it has been difficult or impossible to determine the actual human requirements for a number of trace elements. In addition, the trace elements found in foods are exposed to a number of variables that affect their level of absorption and availability. For example, Cu absorption is depressed by the presence of Cd, Zn, and phytates in the diet. For a number of trace elements, such as Sr, Ti, and Ba, there remains uncertainty as to how, and how much of the actual available element is absorbed. In this study, as well, it is equivocal whether or not the trace element contaminants infused are actually in a form that may cause any biological injury. There have been few extensive studies looking at the trace element intake of North American diets, and the ones that have been performed have used a variety of methods of data collection. The difficulty of analyzing trace elements without introducing extraneous forms of contamination must be appreciated.

Every TPN solution compounded represents an individual pharmaceutical system with its own characteristics. The characteristics may change as the solution is stored for prolonged periods, or is exposed to various plastic materials and temperature conditions. As the number of TPN components increases, the likelihood of creating conditions unfavourable for the stability of one or more of them also increases. The complex composition of TPN solutions makes them highly susceptible to compatibility problems. To provide all nutrients intravenously, TPN solutions must contain a large number of components in a relatively small volume of fluid. The possibility of losses due to component interactions, adsorption and microprecipitation, and gains due to leeching are quite high. Losses of trace elements have been historically attributed to precipitation or adsorption effects. Much of the literature contains confounding results with regard to trace element precipitation from TPN solutions. Many studies have looked at precipitation of trace elements under extreme concentrations of various additives that would not likely be found in TPN solutions [12,39]. Additionally, precipitation was often not confirmed by analysis, rather, visual examinations were made. Zinc, Cu, Mn, Cr [22], and Se [13] were not found to precipitate from standard TPN solutions delivered 24 to 48 hours after compounding and storage in polyvinyl chloride TPN bags at room temperature. Conclusions were based on adequate quantitative recovery of trace elements compared to amounts initially measured in the TPN bags. Elemental precipitates have been suggested to be too small to be seen by the naked eye [39]. X-ray energy dispersive spectroscopy (EDS) linked to an electron microscope was used to examine microprecipitates on membrane filters for the presence of trace elements. The surface of a membrane filter through which a standard filtered TPN solution containing electrolytes and trace element additives (Zn, Cu, Mn, Cr) at concentrations recognized as compatible had passed, showed no evidence of precipitating elements when analyzed [39].

Chelating agents, known to bind or precipitate trace elements such as Zn and Cr out of solution, were not present in any of the component solutions used in this study. Therefore, any reduction in trace elements observed could not be attributed to this group of additives. In this study, no precipitates were observed. However visual detection was hindered by the yellow colour of the MVI additive in the solution and the white base of the in-line filter.

The various studies measuring the recovery of delivered trace elements appear to indicate little or no adsorption to polyvinyl chloride TPN bags, administration tubing, or in-line filters. It was concluded that Zn, Cu, Mn [22,40], Cr [22], and Se [13] were not adsorbed to polyvinyl chloride TPN bags or administration tubing from typical TPN solutions delivered 24 to 48 hours after compounding and storage at room temperature. Zinc, Cu, Cr, Mn, and I were recoverable from TPN solutions after storage in polyvinyl chloride bags at room temperature and refrigerated temperatures up to 24 hours after compounding [20]. Manganese was not significantly changed in four different TPN solutions stored for up to 14 days after compounding into polyvinyl chloride bags and storage at 4°C [41]. Zinc and Cr concentration remained unchanged after prolonged storage in polyvinyl chloride TPN bags for 60 days under refrigeration [9].

Of the recovery studies performed to date no detectible losses in the concentration of Zn, Cu, Mn and Cr from 0.22μ membrane filters [9,22], Zn, Cu and Mn from 0.22μ nylon filters [9] or 0.45μ membrane filters [40], and Se from 0.22μ (unspecified) filters [13] have been reported after storage at room temperature and delivery 36 to 48 hours after compounding. Whether or not the in-line filter adsorbs or traps trace elements after solutions have been subjected to longer storage times or reduced temperature storage is largely unknown. Chromium and Zn in a typical solution of amino acids, dextrose and electrolytes did not adsorb to 0.22μ membrane filters after storage for 60 days under refrigeration [9]. Unfortunately, no authors have looked for the adsorption of any other trace elements (eg, Al, B) by the filter in use. The filters used in the aforementioned experiments were different than the one used in this study (a polyethylene 0.22μ depth filter). To the knowledge of this author, no reports have appeared in the literature which have formally evaluated trace element adsorptive losses by polyethylene in-line filters. Therefore, the question of whether or not trace elements are lost in any significant amount from polyethylene in-line filters remains one for investigation.

The adsorptive behaviours of trace elements depends on a number of factors which, when taken together, make adsorption losses difficult to predict: the form and concentration of trace element, characteristics of the solution (ie, pH, dissolved materials, complexing agents, dissolved gases like oxygen which may influence the oxidation state, and suspended matter); properties of the material (ie, chemical composition, surface roughness and surface area), and external factors (ie, temperature, contact time, access of light, and occurrence of agitation). It is evident that identifying adsorption reactions in delivered TPN solutions is not a simple task. Unfortunately, in the few clinical adsorption studies attempted, the numerous variations in the solutions and storage conditions make prediction of adsorption effects nearly impossible. Additionally, no studies have specifically examined the adsorption characteristics of polyvinyl chloride which is used extensively in TPN delivery and storage systems. Carefully designed studies are necessary with medical grade plastics in order to determine whether or not adsorption of trace elements is a significant problem in TPN delivery systems.

The lack of observed precipitation or adsorption of trace elements in the literature is partially due to the fact that the results quoted were in studies which were not designed to specifically measure precipitation or adsorption reactions. Results were, therefore, largely based upon conjecture. In this study, the potential reactions occurring can only be inferred based upon past studies and the little information available on trace element interactions in TPN solutions. The results of our study appear to indicate that some trace element losses occur in TPN stored for up to one month prior to delivery. However, considering that recommended levels of essential trace elements are delivered despite storage for approximately one month at either 4°C or 20°C, these reactions appear to have minor influences.

Trace element contamination of TPN solutions has been attributed to a number of sources, including contaminated component solutions, leeching or elution from the plastic components in contact with the solution, and particulate matter. It is unlikely that elution of trace element residues from the plastic components was a factor in increasing the levels of trace elements in this study. If trace elements were eluted, there would have been a larger quantity collected earlier during the delivery due to the initial wash-out. However, the experiment performed to assess the stability of trace elements during delivery revealed that there was no change in trace elements over the ten hour delivery period. Particulates were not visually detected in the TPN solutions of this study. However, the addition of the bright yellow MVI additive made it difficult to discern the presence of any particulates. There have been no extensive experiments performed for the sole purpose of revealing trace element contaminants derived from the delivery system. Therefore, the contribution of the delivery system to trace element contamination has remained largely unknown. Some of the reason for this was the lack of technology available during the time of earlier experimentation. Using the ICP-MS in this study, virtually all elements in the periodic table were scanned in samples of delivered solutions to specifically identify and measure existing trace element contaminants. This represents an extraordinary step forward in the analysis of essential or contaminating trace elements in TPN solutions when compared to the past individual trace element determinations performed with atomic absorption spectrometry.

Both essential and nonessential trace elements can become toxic at high concentrations. Unfortunately, very little is known about the intravenous toxicity of most trace elements. Any element infused in excess of the parenteral or orally absorbed amount is a potential candidate for toxicity. The main source of the trace element contamination of TPN solutions still appears to be from the component solutions. However, this study is the first to reveal that significant amounts of trace elements are added by the delivery system. The additive effect of these two sources, for most elements examined in this study, clearly exceeds the normal ranges of absorbed elements from the oral diet. The increased contaminants observed with delivery (B, V, Ti, Co, Sr) present a new problem for investigation. Their sources need to be confirmed. Since there is so little known about the functions and biological effects of most trace elements, the significance of delivering intravenous contaminants such as Ti, Ba, V, Sr, and Co is not known, especially at some of the elevated levels demonstrated in this study. This becomes important for the long-term patient who receives exclusive parenteral support for many years.

The trace elements in this study have been discussed in groups according to their behaviour after delivery. However, there appears to be no consistent chemical connection between any of the elements in the groups, in terms of atomic mass, electron density, reaction characteristics or molecular structure. If this study were repeated with other lots of TPN component solutions, it is possible that different contaminants at different concentrations would be observed due to lot variations. Whether or not the trace elements observed in this study would behave similarly (ie, increase or decrease over time) under the same temperature and storage conditions is open to question.

The National Co-ordinating Committee on Large Volume Parenterals (NCCLVP) [42] has made general recommendations that TPN solutions should be administered immediately after compounding. This is because of the fact that limited data on trace element stability and compatibility has been available only up to 48 hours after the addition of the trace elements to the solutions. Such conditions are not feasible for the home TPN patient. However, the results of our study have shown that the essential trace elements, Zn, Cu, Mn, and Se are delivered at recommended levels in TPN solutions stored up to 1 month at 4 or 20°C; Cr remains in question. Grant [43] also recommends that for home TPN patients, solutions should be stored for no longer than 30 days under refrigeration. It must be noted that trace element contaminants present in the TPN component solutions may have assisted in maintaining the adequate levels upon delivery by buffering the effects of reactions occurring to reduce the trace element content over time.

CONCLUSION

Since losses of trace elements in TPN solutions may contribute to a suboptimal nutritional status, and delivery of excess amounts may induce toxicity, it is of practical importance to determine the amounts of trace elements received by patients administered TPN. However, the number of possible TPN solution combinations are enormous since solutions are designed to be patient-specific. This makes the potential number and types of reactions which could occur in TPN solutions staggering. Therefore, it is difficult to make definitive conclusions about the delivered trace element content of "TPN solutions" as a single entity. There has been little scientific data generated over the years on the stability and compatibility of trace elements due to the lack of technology. Assumptions that solutions are stable (no trace elements are lost or gained) since no precipitate develops or no adverse effects have occurred in the patients as a result of delivery are dangerously presumptive. Presently, the general lack of knowledge and analytical technology surrounding trace elements makes it difficult to interpret the significance of the findings in the studies performed to date. It is hoped that as a result of further research, the various reactions within TPN delivery systems can be confirmed, and that ranges of added TPN components can be set where there is minimum or maximum interference with delivery of trace elements. However, this can only be achieved after the exact amount of trace elements delivered are known, ie, after the removal or standardization of trace element contaminants in TPN solution components. More work on trace elements in a delivery setting is required in order to determine whether or not the types of delivered elements and quantities entering the patient on a daily basis present cause for concern.

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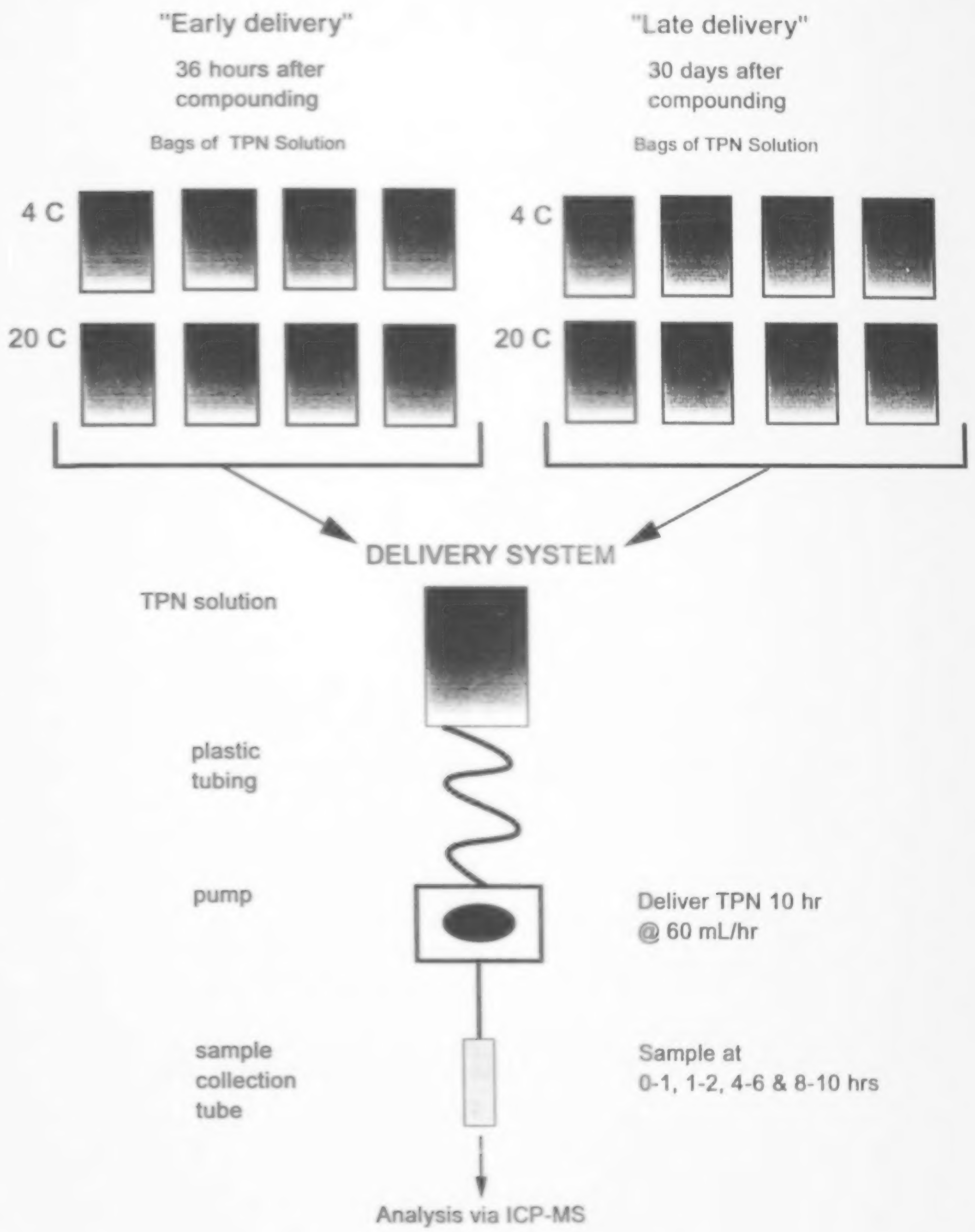
"Early delivery"
36 hours after compounding
Bags of TPN Solution
"Late delivery"
30 days after compounding
Bags of TPN Solution
4 C
20 C
4 C
20 C
DELIVERY SYSTEM
TPN solution
plastic tubing
pump
Deliver TPN 10 hr @ 60 mL/hr
sample collection tube
Sample at 0-1, 1-2, 4-6 & 8-10 hrs
Analysis via ICP-MS

Figure 5-1. Outline of delivery experiment. Each bag underwent delivery as described at respective times/temperatures.

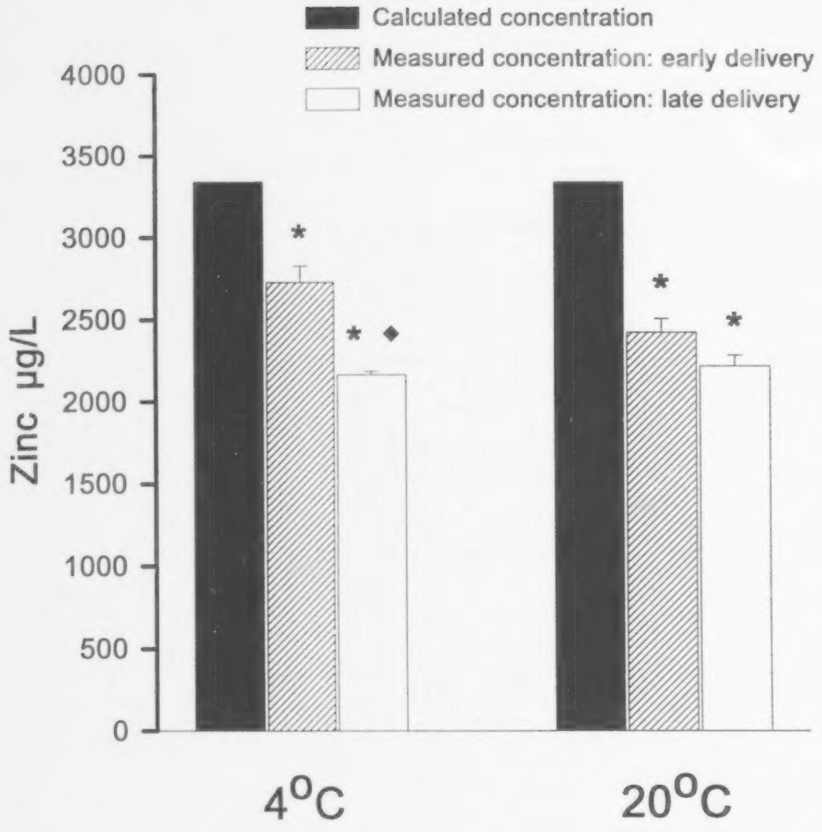

Figure 5-2. Concentration of zinc [Zn] (µg/L) delivered in a TPN solution early (36 hours) (diagonal bars) and late (30 days) (open bars) after storage at 4°C or 20°C. Solid bars represent total Zn calculated (sum of intentional additive and contamination from Lot 1 components). At 4°C: [Zn] at early and late delivery less than calculated; *$p<0.0008$, $p<0.0001$, respectively, and [Zn] at late delivery less than early delivery; •$p<0.001$. At 20°C: [Zn] at early and late delivery less than calculated; *$p<0.0001$. Results expressed as mean ± SEM (n=4 TPN bags per time/temperature combination).

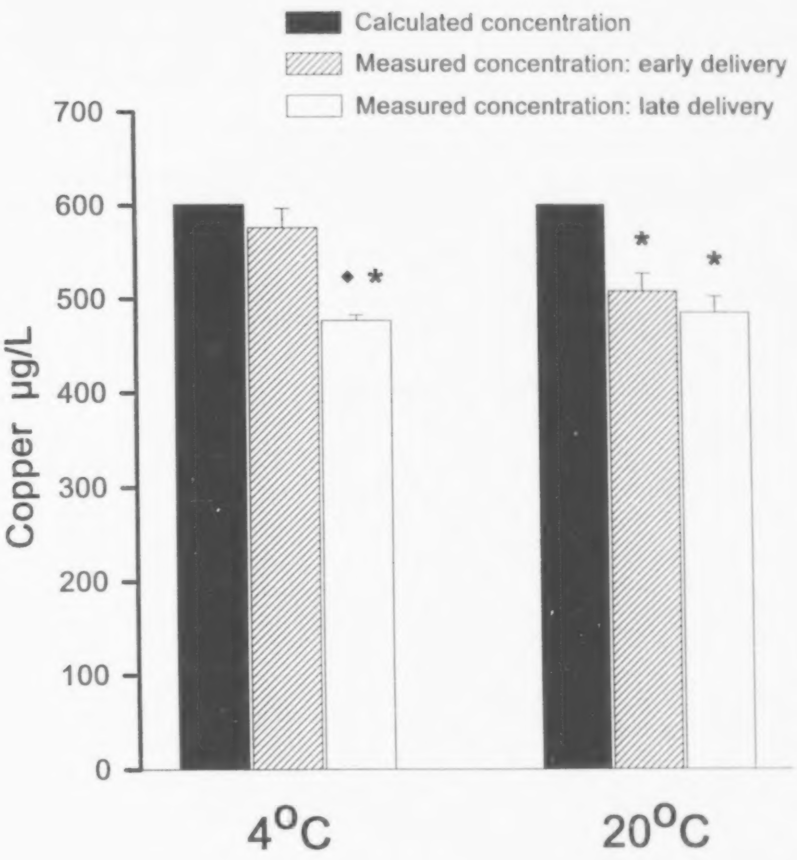

Figure 5-3. Concentration of copper [Cu] (µg/L) delivered in a TPN solution early (36 hours) (diagonal bars) and late (30 days) (open bars) after storage at 4°C or 20°C. Solid bars represent total Cu calculated (sum of intentional additive and contamination from Lot 1 components). At 4°C: [Cu] at late delivery less than early delivery (•$p<0.004$) and calculated (*$p<0.0001$). At 20°C: [Cu] at early and late delivery less than calculated ; *$p<0.003$, $p<0.0006$, respectively. Results expressed as mean ± SEM (n=4 TPN bags per time/temperature combination).

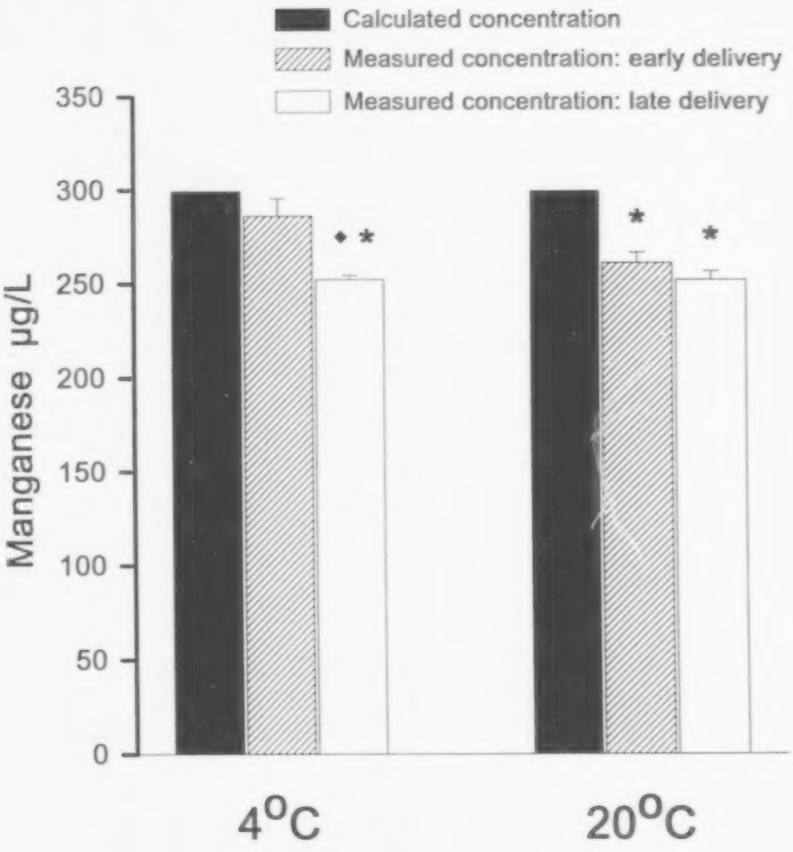

Figure 5-4. Concentration of manganese [Mn] (µg/L) delivered in a TPN solution early (36 hours) (diagonal bars) and late (30 days) (open bars) after storage at 4°C or 20°C. Solid bars represent total Mn calculated (sum of intentional additive and contamination from Lot 1 components). At 4°C: [Mn] at late delivery less than early delivery (◆$p<0.01$) and calculated (*$p<0.0001$). At 20°C: [Mn] at early and late delivery less than calculated; * $p<0.0005$, $p<0.0001$, respectively. Results expressed as mean ± SEM (n=4 TPN bags per time/temperature combination).

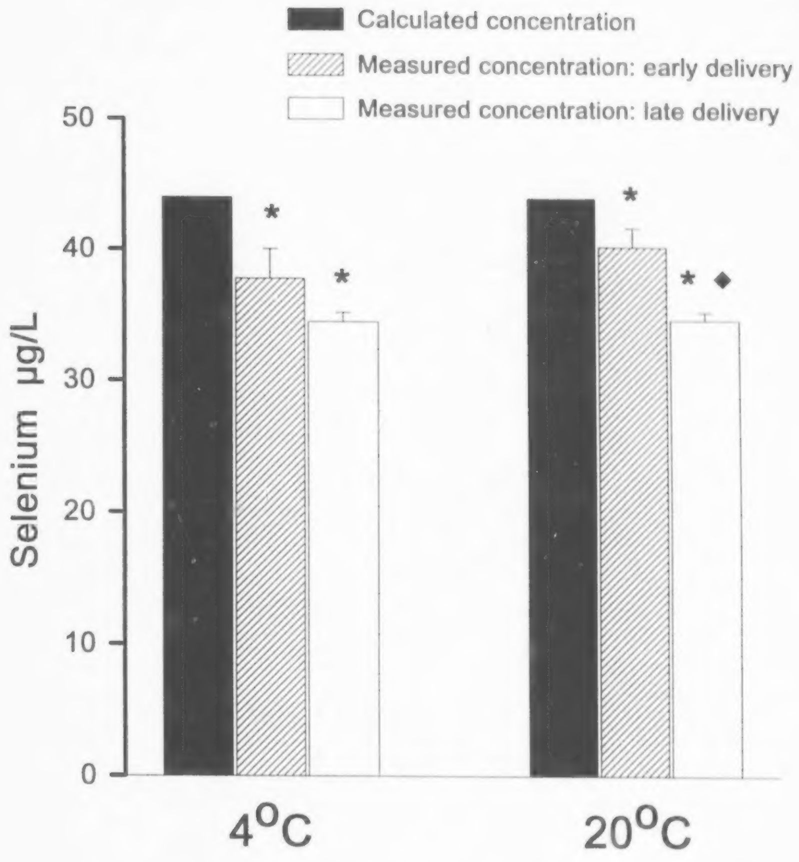

Figure 5-5. Concentration of selenium [Se] (µg/L) delivered in a TPN solution early (36 hours) (diagonal bars) and late (30 days) (open bars) after storage at 4°C or 20°C. Solid bars represent total Se calculated (sum of intentional additive and contamination from Lot 1 components). At 4°C: [Se] at early and late delivery less than calculated; *$p<0.03$, $p<0.0001$, respectively. At 20°C: [Se] at early and late delivery less than calculated; *$p<0.04$, $p<0.0001$, respectively, and [Se] at late delivery less than early delivery •$p<0.01$. Results expressed as mean ± SEM (n=4 TPN bags per time/temperature combination).

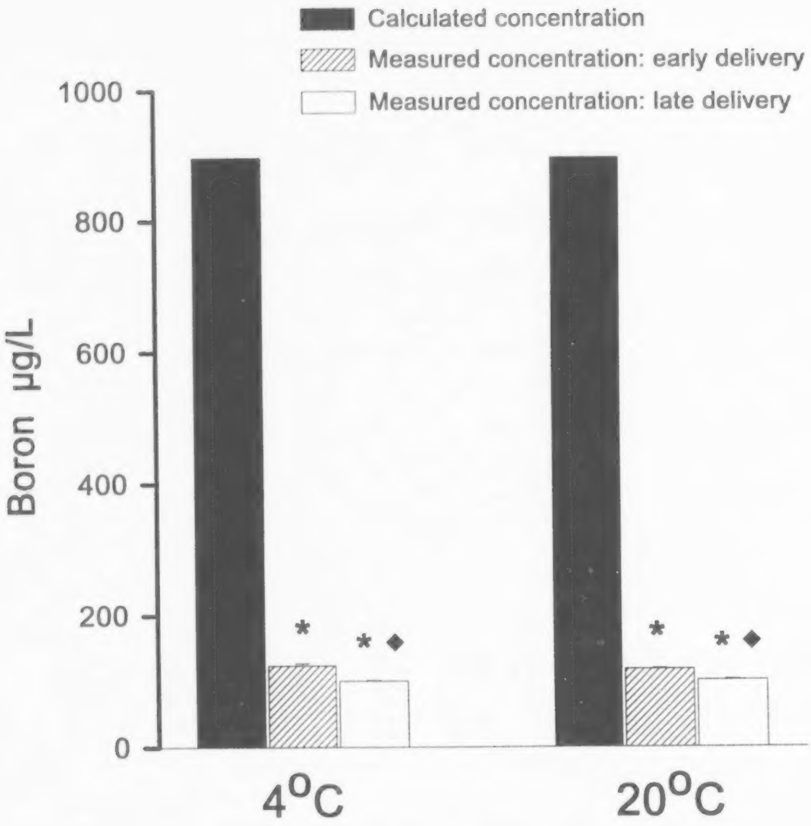

Figure 5-6. Concentration of boron [B] (µg/L) delivered in a TPN solution early (36 hours) (diagonal bars) and late (30 days) (open bars) after storage at 4°C or 20°C. Solid bars represent total B calculated (sum of contamination from Lot 1 components). At 4°C: [B] at early and late delivery less than calculated; *p<0.0001, and [B] at late delivery less than early delivery; •p<0.0005. At 20°C: [B] at early and late delivery less than calculated; *p<0.0001, and [B] at late delivery less than early delivery; •p<0.0001. Results expressed as mean ± SEM (n=4 TPN bags per time/temperature combination).

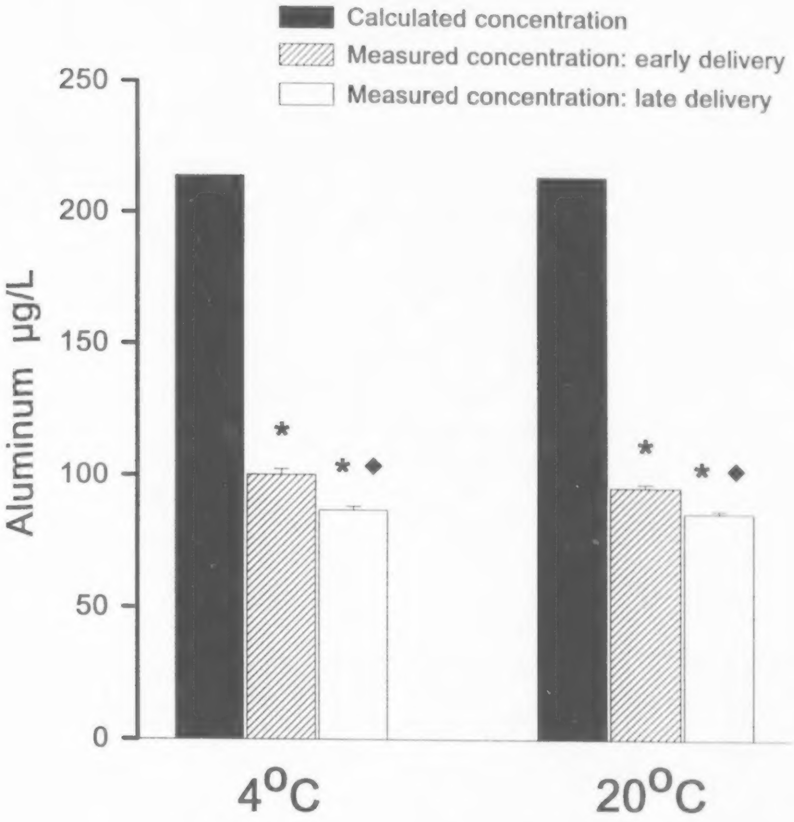

Figure 5-7. Concentration of aluminum [Al] (µg/L) delivered in a TPN solution early (36 hours) (diagonal bars) and late (30 days) (open bars) after storage at 4°C or 20°C. Solid bars represent total Al calculated (sum of contamination from Lot 1 components). At 4°C: [Al] at early and late delivery less than calculated; *$p<0.0001$, and [Al] at late delivery less than early delivery; ◆$p<0.002$. At 20°C: [Al] at early and late delivery less than calculated; *$p<0.0001$, and [Al] at late delivery less than early delivery; ◆$p<0.002$. Results expressed as mean ± SEM (n=4 TPN bags per time/temperature combination).

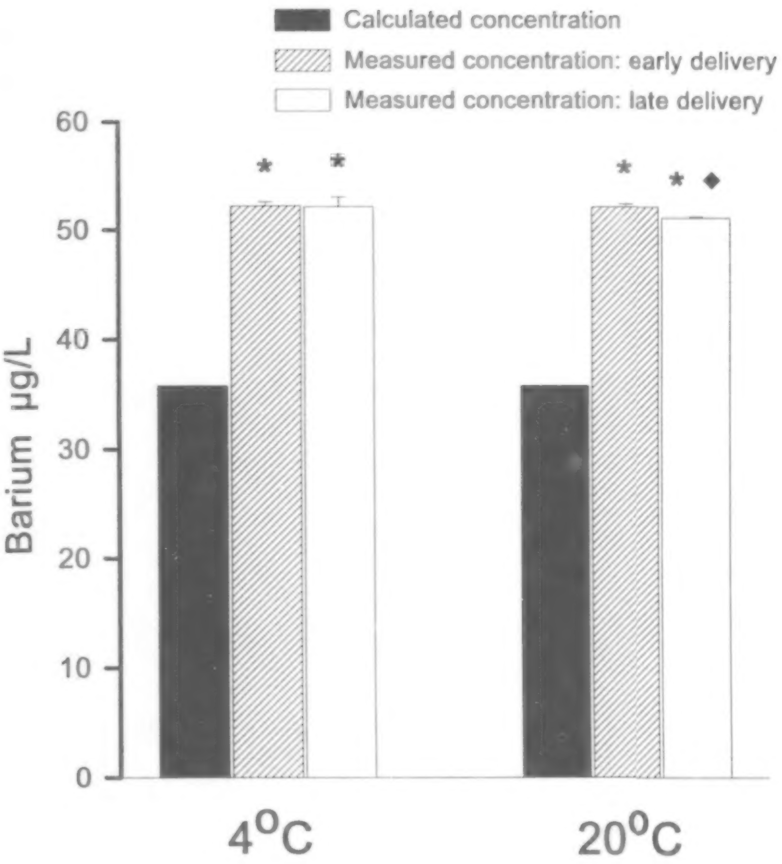

Figure 5-8. Concentration of barium [Ba] (µg/L) delivered in a TPN solution early (36 hours) (diagonal bars) and late (30 days) (open bars) after storage at 4°C or 20°C. Solid bars represent total Ba calculated (sum of contamination from Lot 1 components). At 4°C: [Ba] at early and late delivery greater than calculated; $^{*}p<0.0001$. At 20°C: [Ba] at early and late delivery greater than calculated; $^{*}p<0.0001$, and [Ba] at late delivery less than early delivery; •$p<0.02$. Results expressed as mean ± SEM (n=4 TPN bags per time/temperature combination).

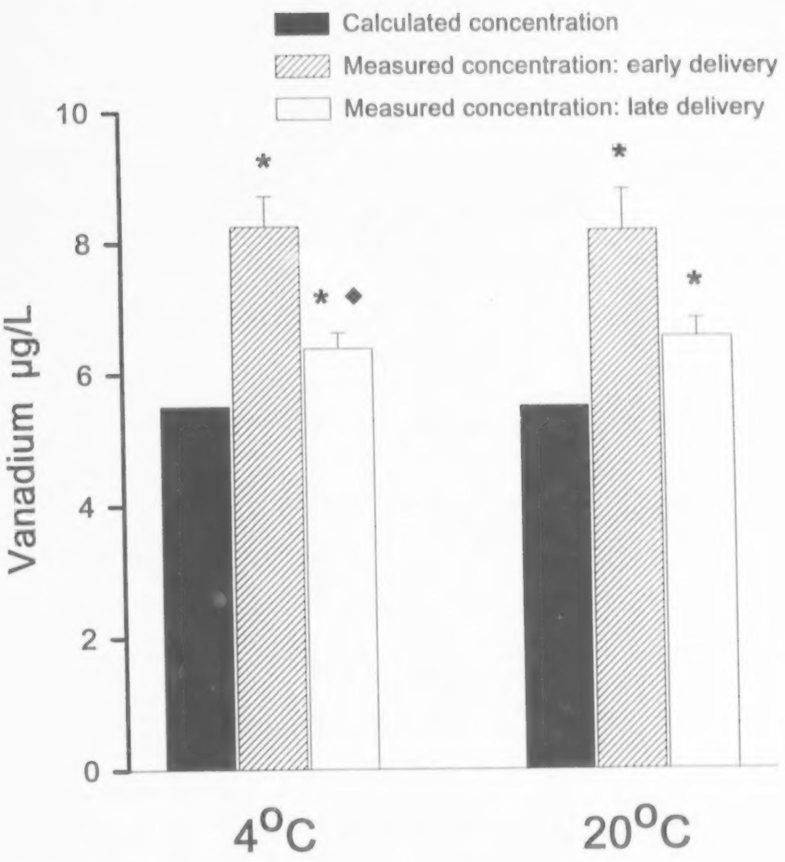

Figure 5-9. Concentration of vanadium [V] (µg/L) delivered in a TPN solution early (36 hours) (diagonal bars) and late (30 days) (open bars) after storage 4°C or 20°C. Solid bars represent total V calculated (sum of contamination from Lot 1 components). At 4°C: [V] at early and late delivery greater than calculated; *p<0.0009, p<0.01, respectively, and [V] at late delivery less than early delivery; •p<0.01. At 20°C: [V] at early and late delivery greater than calculated; *p<0.005, p<0.009, respectively. Results expressed as mean ± SEM (n=4 TPN bags per time/temperature combination).

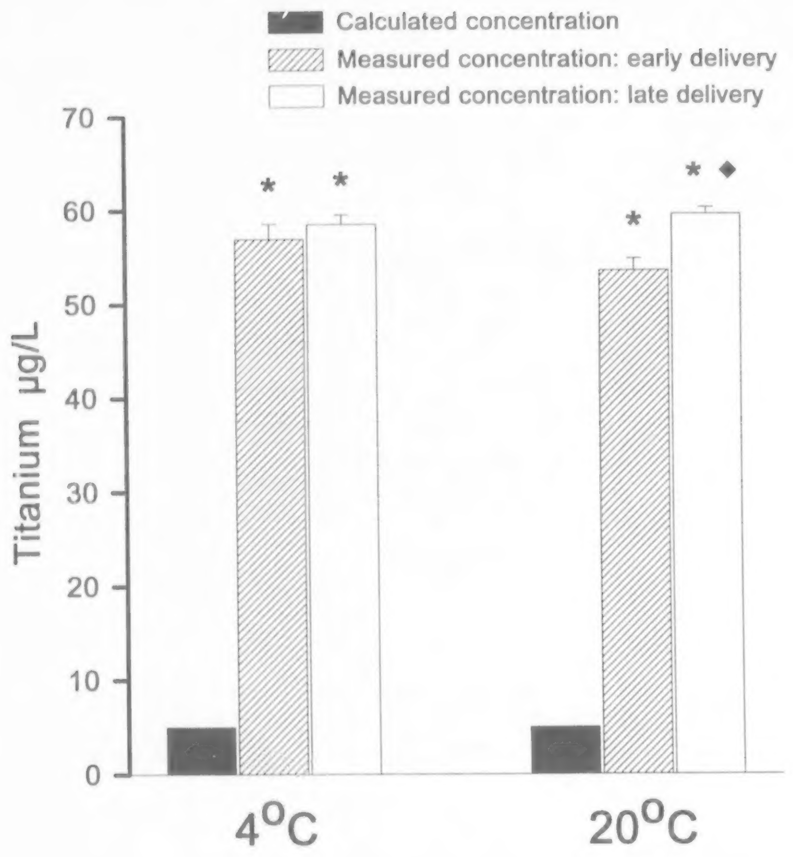

Figure 5-10. Concentration of titanium [Ti] (µg/L) delivered in a TPN solution early (36 hours) (diagonal bars) and late (30 days) (open bars) after storage at 4°C or 20°C. Solid bars represent total Ti calculated (sum of contamination from Lot 1 components). At 4°C: [Ti] at early and late delivery greater than calculated; *p<0.0001. At 20°C: [Ti] at early and late delivery greater than calculated;*p<0.0001, and [Ti] at late delivery greater than early delivery; ◆p<0.006. Results expressed as mean ± SEM (n=4 TPN bags per time/temperature combination).

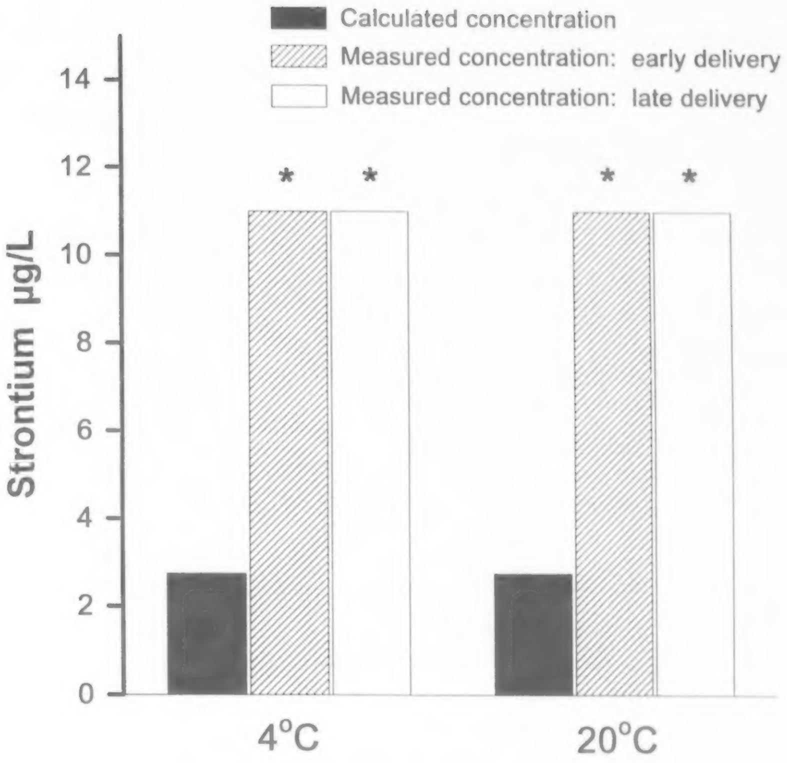

Figure 5-11. Concentration of strontium [Sr] (µg/L) delivered in a TPN solution early (36 hours) (diagonal bars) and late (30 days) (open bars) after storage at 4°C or 20°C. Solid bars represent total Sr calculated (sum of contamination of Lot 1 components). At 4°C: [Sr] at early and late delivery greater than calculated; *$p<0.0001$. At 20°C [Sr] at early and late delivery greater than calculated; *$p<0.0001$. Results expressed as mean ± SEM (n = 4 TPN bags per time/ temperature combination).

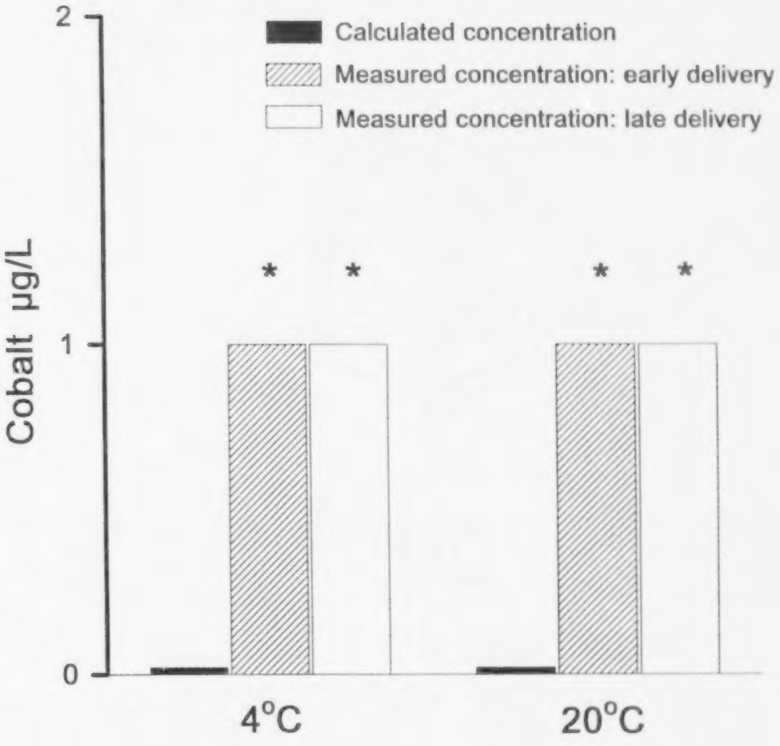

Figure 5-12. Concentration of cobalt [Co] (µg/L) delivered in a TPN solution early (36 hours) (diagonal bars) and late (30 days) (open bars) after storage at 4°C or 20°C. Solid bars represent total Co calculated from Lot 1 components (ie, 0 µg/L). At 4°C: [Co] at early and late delivery greater than calculated; *p<0.0001. At 20°C [Co] at early and late delivery greater than calculated; *p<0.0001. Results expressed as mean ± SEM (n=4 TPN bags per time/ temperature combination).

Table 5-1. TPN solution used in the early and late delivery experiment

Solution	Added Volume (mL)
NaCl 4mM/mL	11.66
KCL 2mM	22.22
Travasol 10% without electrolytes	300.00
MTE-6 trace element concentrate[1]	0.56
Dextrose 70%	257.14
Ca-gluconate 10%	19.16
MVI adult multivitamin[2]	5.56
$MgSO_4$ 50%	2.18
Sterile water	381.52
Total	1000.00

[1] MTE-6 concentrate contains per mL: 5 mg Zn, 1 mg Cu, 0.5 mg Mn, 10 μg Cr, 60 μg Se, 75 μg I.

[2] MVI adult contains per 10 mL: vitamin C 1000 mg, vitamin A 10 000 IU, vitamin D 1000 IU, vitamin E (dl-alpha tocopheryl acetate) 10 IU, thiamine (as hydrochloride) 45 mg, riboflavin (as sodium phosphate) 10 mg, niacinamide 100 mg, pyridoxine (as hydrochloride) 12 mg, d-pantothenic acid (as d-panthenol) 26 mg, benzyl alcohol 9 mg.

Table 5-2. Trace elements examined with the "Spectrum-directed" ICP-MS screen

Li (Lithium)	Ni (Nickel)	Zr (Zirconium)	Te (Tellurium)	Gd (Gadolinium)	Re (Rhenium)
Be (Beryllium)	Cu (Copper)	Nb (Niobium)	I (Iodine)	Tb (Terbium)	Os (Osmium)
B (Boron)	Zn (Zinc)	Mo (Molybdenum)	Xe (Xenon)	Dy (Dyprosium)	Ir (Iridium)
Al (Aluminum)	Ga (Gallium)	Ru (Ruthenium)	Cs (Cesium)	Ho (Holmium)	Pt (Platinum)
Sc (Scandium)	Ge (Germanium)	Rh (Rhodium)	Ba (Barium)	Er (Erbium)	Au (Gold)
Ti (Titanium)	As (Arsenic)	Pd (Palladium)	La (Lanthanum)	Tm (Thulium)	Hg (Mercury)
V (Vanadium)	Se (Selenium)	Ag (Silver)	Ce (Cerium)	Yb (Ytterbium)	Tl (Thallium)
Cr (Chromium)	Kr (Krypton)	Cd (Cadmium)	Pr (Praseodymium)	Lu (Lutetium)	Pb (Lead)
Mn (Manganese)	Rb (Rubidium)	In (Indium)	Nd (Neodymium)	Hf (Hafnium)	Bi (Bismuth)
Fe (Iron)	Sr (Strontium)	Sn (Tin)	Sm (Samarium)	Ta (Tantalum)	Th (Thorium)
Co (Cobalt)	Y (Yttrium)	Sb (Antimony)	Eu (Europium)	W (Tungsten)	U (Uranium)

Table 5-3. Comparison of dietary intakes and intravenously delivered concentrations of trace elements stored at various time/temperature combinations.

TRACE ELEMENT	AVERAGE DIETARY INTAKE (North America) µg/day	ABSORPTION FACTOR %	AVERAGE AMOUNT ABSORBED µg/day	RECOMMENDED PARENTERAL DELIVERY µg/day	EXPECTED AMOUNT DELIVERED µg/day[1]				PERCENT OF AVERAGE DAILY ABSORPTION[2]
					4°C		20°C		
					Early	Late	Early	Late	
Zn	10 000[3]	40[3]	4 000	2 500 to 4 000[11]	5 458	4 332	4 850	4 438	119
Cu	1 600 to 2 100[3]	40[3]	640 to 840	500 to 1 500[11]	1 152	954	1 018	972	138
Mn	3 000 to 3 800[3]	3 to 8[3]	165 to 209	150 to 800[11]	572	504	520	504	280
Se	100 to 200[3]	80[3]	80 to 160	40 to 120[12]	76	70	80	70	62
Cr	56 to 282[3]	0.5 to 2[3]	0.7 to 3.5	10 to 15[11]					
B	2 000 to 4 000[3]	30 to 90[4]	900 to 2 700	——	248	202	236	204	12
Al	20 000[3]	< 1[5]	200	——	200	174	192	172	93
Ba	600 to 900[6]	6[4]	36 to 54	——	104	104	104	102	231
V	10 to 30[3]	0.1 to 1[7]	0.06 to 0.165	——	16	12	16	14	13274
Ti	100 to 1 000[8]	1 to 3[8,9]	2 to 20	——	114	118	108	120	1045
Sr	590 to 2280[4]	5 to 25[4]	89 to 342	——	22	22	22	22	10
Co	5 to 45[10]	25[10]	1 to 11	——	2	2	2	2	33

[1] Concentration of trace elements delivered in 2 litres of experimental TPN solution per day. Expected amount delivered assuming the occurance of any additions or losses. Early delivery = 36 hours after compounding; Late delivery = 30 days after compounding. Values for Cr not reported.

[2] Based on average of all delivery periods, with the exception of Cr which was not reported due to organic interferences with the ICP-MS.

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CHAPTER 6

SUMMARY AND CONCLUSION

This study tested the hypothesis that the amount of trace element initially thought to be present in a home TPN solution is different than the amount delivered to a patient at the time of infusion. The supposition was based upon research which has revealed trace element deficiencies and excesses in long-term TPN patients despite delivery of what has been thought to be adequate amounts of trace elements. The literature contains numerous accounts of trace element contamination of TPN component solutions which confound the expected levels of trace elements thought to be provided to the patient. Additionally, studies have shown that various plastic, glass, and metal materials are capable of adsorbing or leeching trace elements, thereby altering the amounts present in the original solutions. Many of these materials have been found to be in contact with TPN solution at various points in its production. Hence, we proposed that trace element alterations occurred in home TPN solutions and aimed to detrmine the degree of alteration in a typical solution subjected to clinically relevant variables.

The three studies designed around the three areas of TPN production (components, compounding, and delivery) have confirmed our hypothesis that the delivered trace element concentrations are altered from the levels intended for patients. In chapter 3 we confirmed the presence of unexpected and variable trace element contaminants in TPN components, which were dependent upon the lot and manufacturer investigated. In chapter 4, we revealed the addition of trace element (ie, Zn) upon passage of the sterile water component through compounding devices and questioned the further addition (or potential adsorption) of trace element contaminants with the passage of other individual TPN components through the system. Finally, in Chapter 5 we demonstrated that the delivery of TPN solution under clinically relevant conditions resulted in both addition (Ba, V, Ti, Sr, Co) and losses (Zn, Cu, Mn, Se, B, Al) of trace elements likely resulting from various leeching, or precipitation and adsorption reactions, respectively. Although some loss of trace elements did occur in TPN solutions stored up to 30 days at either 4 or 20° C prior to delivery, the essential added trace elements (Zn, Cu, Mn, and Se; Cr remains uncertain) delivered remained within the American Medical Association recommended levels for parenteral infusion. These "reduction" reactions (ie, precipitation and adsorption) thus appeared to have minor influences. However, it should be noted that the contaminanted TPN components may have helped cushion some of the losses incurred by compounding or delivery of the complete solution.

This study raises clinical concerns regarding the trace element contaminants (Al, Ba, V, Ti) appearing at delivered levels which were considerably greater than those absorbed from the daily oral diet. The fact that TPN bypasses the normal barriers of the gastrointestinal tract places an immense responsibility on the part of health care providers to ensure that potentially harmful trace elements are not delivered. Due to the paucity of information surrounding trace elements in general, the significance of such contaminants in delivered home TPN solutions can not be determined at this time. A summary of the trace element alterations (in Lot 1 components) appearing as a result of exposure to the three areas of TPN production appears in Table 6-1. This table demonstrates the inadequate information regarding the potential trace element alterations occurring during compounding, and the specific site (bag, tubing,filter) of alterations during delivery.

It is apparant from this study that all three areas of TPN production merit further exploration. The inadequate technology in the past made extensive coverage of trace element contaminants of TPN components a near impossibilty. It would, therefore, be beneficial to analyze other TPN components, including lipid, with the mulitielemental methods available to further comment on the extent of trace element contamination in TPN components. In order to determine where the losses and additions of trace elements occur after delivery of complete home TPN solutions subjected to typical clinical time and temperature variables, it appears necessary to examine both the compounding and delivery systems in more detail. An experiment examining trace element additions (from leeching reactions) or losses (from adsorption reactions) provoked by the passage of each separate component through the plastic tubing of the compounding device would confirm whether or not any trace element alterations transpire during compounding. In conjunction, another experiment simultaneously measuring trace element concentration changes at various delivery points in a clinically relevant system exposed to typical time and temperature variables (ie, simultaneous sampling from the TPN bag, pre-filter solution, and post-filter solution) would provide information regarding the source of the trace element contaminants which appear to increase during delivery (Ba, V, Ti, Sr and Co). Changes to the delivery and compounding systems, to reduce or exclude the delivery of these elements for the long-term home TPN patient,deserve consideration until the significance of these delivered elements can be further clarified.

Table 6-1. Summary of trace element alterations during compounding and delivery compared to expected concentrations in one litre of TPN solution based on Lot 1 components.

Element	Expected concentration μg/L[1]	Cumulative component concentration μg/L[2]	Compounding + / - / ?[3]	Measured concentration after delivery μg/L[4]
Zn	2780	3341(+)	+	2384.7(-)
Cu	560	601(+)	?	512.0(-)
Mn	280	299(+)	?	262.8(-)
Cr[5]	5.56	11 to 15(+)	?	5.86(-)
Se	33.33	44(+)	?	36.8(-)
B	0	897(+)	?	111.3(-)
Al	0	214(+)	?	92.2(-)
Ti	0	5(+)	?	57.9(+)
Ba	0	36(+)	?	51.8(+)
V	0	6(+)	?	7.3(+)
As	0	0(+)	?	0
Sr	0	2.3(+)	?	11(+)
Co	0	0	?	1(+)

[1] Expected concentration of trace elements present as part of 0.56 mL MTE-6 trace element additive. MTE-6 concentrate contains per mL: 5 mg Zn, 1 mg Cu, 0.5 mg Mn, 10 μg Cr, 60 μg Se, 75 μg I.

[2] Total calculated amount of added trace element and contaminants in nine TPN components from Lot 1 used in the typical TPN solution as outlined in Table 3-2, Chapter 3. (+) denotes a concentration greater than the expected concentration.

[3] + = additions, - = reductions, and ? = unknown effect of compounding on trace element alterations in the TPN solution made from Lot 1 components.

[4] Mean of the four time/temperature delivery periods described in Chapter 5, Figure 5-1. The + or - denote additions or losses compared to the cumulative component concentration. The exact site responsible for the trace element alterations (TPN bag, tubing, filter) has not been defined.

[5] Cr results reflect an estimate of values based on correction factor derived from GFAAS (as described in Chapter 2) due to problems with interferences during ICP-MS analysis.